Living Resources Committee **ICES CM 2003/G:06 Ref. D**

Report of the

Planning Group on North Sea Cod and Plaice Egg Surveys in the North Sea

Ijmuiden, Netherlands
24–26 June 2003

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer
Palægade 2–4 DK–1261 Copenhagen K Denmark

Section **Page**

1 INTRODUCTION

1.1 Background

The current discussions on recovery plans for depleted fish stocks has highlighted the need for updated information on the extent and timing of spawning. This information can to some extent be estimated from the catches of mature adults in fisheries surveys but more precise information can be provided from egg surveys. This is because spawning may occur in regions which are not accessible to fishing gear; fisheries surveys may not coincide with the peak of spawning; unlike adult fish, eggs do not actively avoid sampling gear and because large numbers of eggs are spawned by each female, egg distributions may give more reliable estimates of spawning extent.

A Scientific Expert Conference related to the Fifth North Sea Conference (Bergen, 20-22 February, 2002) recommended as one of their short-term high priority areas for research that spawning grounds of commercial fish be mapped and monitored. This forms one element of development of an ecosystem based approach to fisheries management. The requirement for this information has also been noted by ICES Regional Ecosystem Study Group for the North Sea (ICES 2003). In response, PGEGGS was established in 2001 by ICES to plan an internationally co-ordinated ichthyoplankton sampling program for the North Sea.

The TOR of PGEGGS focus on two species, cod (Gadus morhua) and plaice (Pleuronectes platessa). The North Sea cod stock is assessed to be outside safe biological limits and SSB is currently at an historic low. The most recent egg survey information on North sea cod spawning grounds dates from the late-1980s and there are indications that these grounds and their relative importance may have changed since that time.

The state of North Sea plaice stock is also poor and updated information on the spawning for this stock is also important. Traditionally, cod and plaice spawn in similar areas and at about the same time. Hence, a common egg survey program will provide information on the spatial and temporal extent of spawning for both species.

1.2 Terms of reference

ICES resolution C.Res. 2002/2D05: The **Planning Group on North Sea Cod and Plaice Egg Surveys** [PGEGGS] (Chair: C.Fox, UK) will meet in IJmuiden from 24-26 June 2003 to:

a) review the results of a trial cruise to be carried out in March 2003 in the Irish Sea for the purpose of testing genetic tools;
b) review the progress of current projects on the identification of cod eggs using genetic probes;
c) plan an international survey to map the distribution of cod and plaice spawning in 2004;
d) develop protocols for evaluating and presenting the data.

PGEGGS will report by 10 July 2003 for the attention of the Living Resources Committee, who will be Parent, and to the Resource Management Committee at the 2003 ASC.

1.3 Participants

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1.4 Acronym

PGEGGS agreed to adopt the acronym PLACES (Plaice and Cod Egg Surveys) for the co-ordinated survey program.

1.5 Status of PGEGGS in relation to Lowestoft meeting, 9 – 11 April 2002

An initial meeting of PGEGGS took place in 2002 with more elaborate terms of reference including fecundity and biomass estimation using egg production methods. A draft report was prepared, this has been made available to PGEGGS members. Sections of this draft report were used as the basis for the 2003 report but modified in light of the more restricted TOR.

2 OVERVIEW OF CURRENT KNOWLEDGE OF COD SPAWNING AND EARLY LIFE HISTORY

2.1 Cod Spawning

Several attempts have been made to define the spawning areas of cod in the North Sea (figure 2.1.1). However, since no fully comprehensive ichthyoplankton surveys have ever been conducted in this region, these efforts have been based upon compilations of existing small-scale survey data and distributions of maturing adults (fisheries surveys and commercial catches).

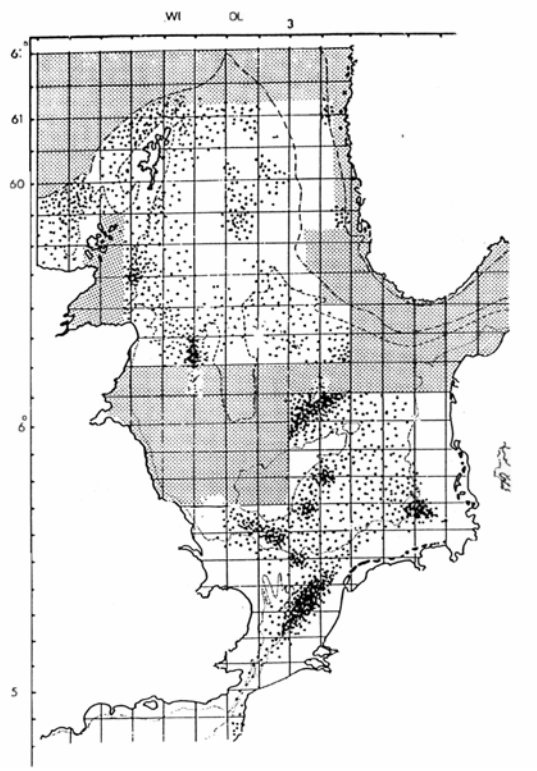

Figure 2.1.1 Spawning areas of cod in the North Sea according to information after 1945. Note that the shaded areas have NOT been surveyed (Daan 1978)

Spawning takes place from the beginning of January through to April. Spawning is perhaps slightly later with increasing latitude, at least around the British Isles (figure 2.1.2).

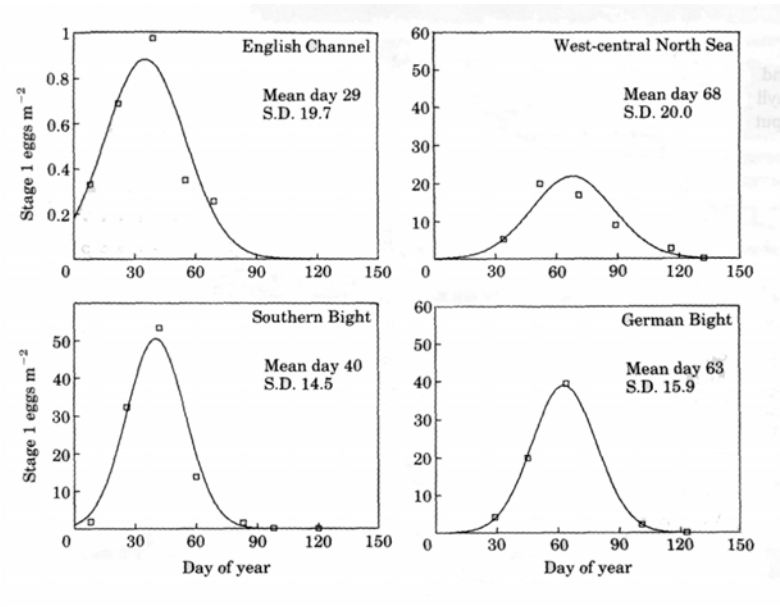

Figure 2.1.2 Abundance of stage I cod eggs and fitted normal distributions with mean dates of spawning and standard deviations. For the English Channel cod eggs of all stages are amalgamated because abundance of stage I eggs was too low to give an estimate (Brander 1994b)

In the past a small amount of spawning has been recorded in the autumn but this is probably not significant (Brander 1994a). Spawning occurs offshore in waters of salinity 34-35 (Riley & Parnell 1984). In the more northern areas spawning may be associated with subterranean banks on which the spawning fish may aggregate. Early surveys were however hampered by the inability to distinguish early stage eggs of cod and haddock. The rate of egg development is mainly related to temperature (Thompson & Riley 1981).

Peaks in egg and larval concentrations were found between the Flamborough and the southwestern flank of Dogger Bank, and another in the Southern Bight off the Dutch coast (Brander 1994a). In addition, further cod spawning is observed at the southeastern edge of the Dogger Bank towards the Horns Reef area (figure 2.1.3). These parts of the southern North Sea are apparently the most important spawning sites.

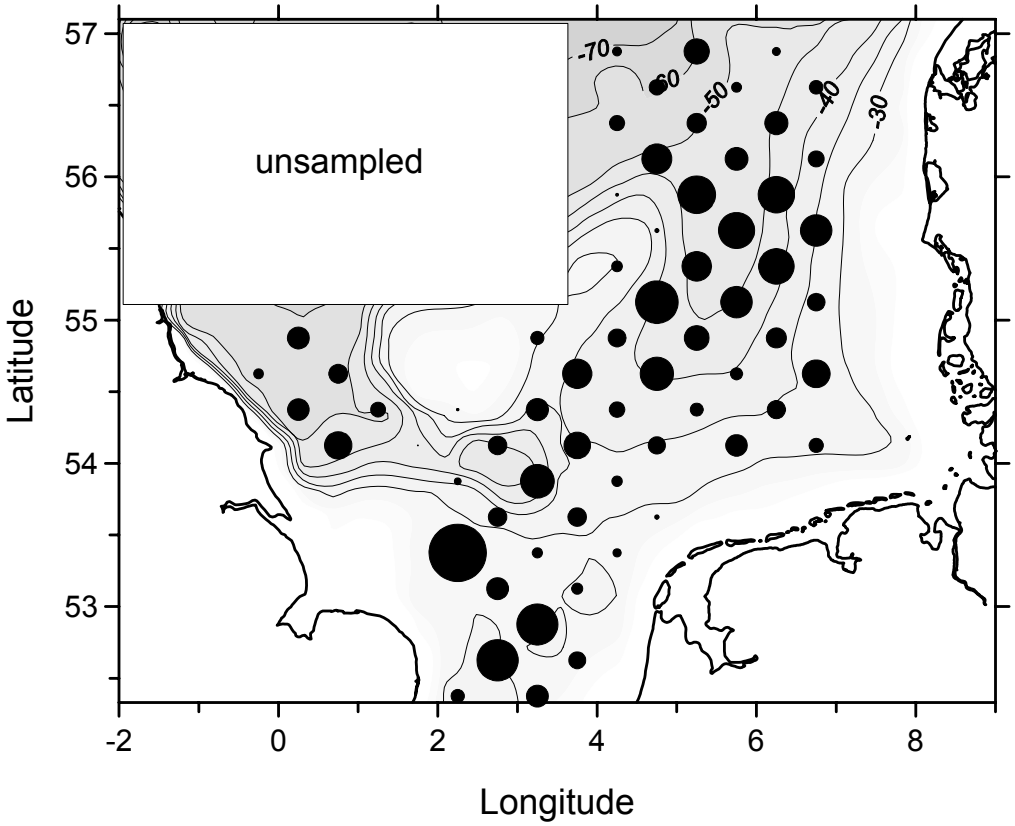

Figure 2.1.3 Distribution of cod eggs based on data from Heessen and Rijnsdorp (1989). Filled symbols are proportional to the accumulated egg production during January-February 1988.

Based on surveys conducted by Harding (1987), cod spawning also occurs off the NE coast of England.

Ichthyoplankton surveys around the coast of Scotland have been undertaken by Raitt (1967), Saville (1959) and Heath (1994). The Saville surveys were principally concerned with haddock but Raitt (1967) re-analysed the data and presented maps for cod. Since they were unable to distinguish early stage cod and haddock eggs the results are based upon the occurrence of late stage eggs. The 1950s surveys in March showed some cod eggs off Butt of Lewis, west of Orkney and Shetland and off the Moray Firth and east Scottish coast. By April, cod eggs were more abundant and cod larvae were common across the survey area. By May the occurrence of cod eggs and larvae was much reduced (figure 2.1.4).

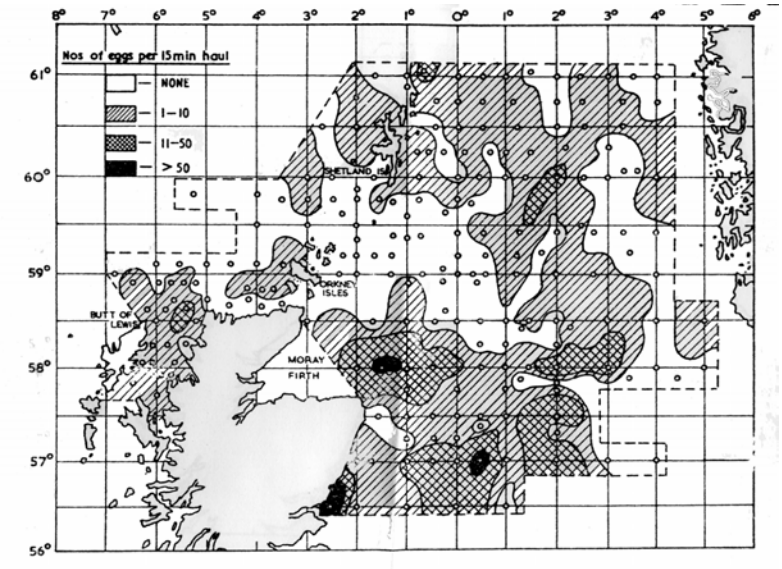

Figure 2.1.4 Cod egg distributions from 1950s surveys (Raitt 1967).

Heath (1994) also conducted ichthypplankton surveys around the Scottish coast in the early 1990s. Based upon the proportions of late stage eggs and larvae, the majority of the cod-like eggs sampled were probably haddock. Positively identified (late stage) cod eggs were only found around Bergen Bank and Orkney (figure 2.1.5).

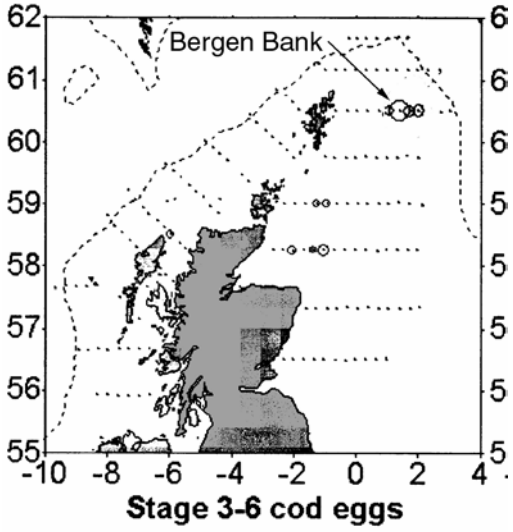

Figure 2.1.5 Occurrence of late stage cod eggs from ichthyoplankton surveys undertaken in 1992 (Heath et al. 1994).

2.2 Cod larval distribution and development

Information on the distribution of early stage cod larvae is available from a study by Munk (2002). The study covered the North Sea from 54˚N to 57˚N, and major concentrations of larvae (up to 2 m^{-2}) were found in the German Bight area and at the southern part of Dogger Bank (see figure 2.2.1). The observations indicate that larval distribution is related to hydrographic characteristics. All larvae were found in water masses influenced by freshwater river-outflow (ROFI areas), and highest concentrations of larvae were seen at the inshore side of the frontal zone between ROFI's and more saline offshore water. Also in later stages (sizes 0.8 – 4 cm) the distribution of cod larvae and juveniles appears linked to frontal hydrography (Munk et al. 1995, Munk et al. 1999). During studies 1992-1994 in the eastern North Sea the cod larvae/juveniles were predominantly seen in the German Bight, around Jutland- , Little Fisher- and Greater Fisher Bank and along the shelf slope into Skagerrak, figure 2.2.2.

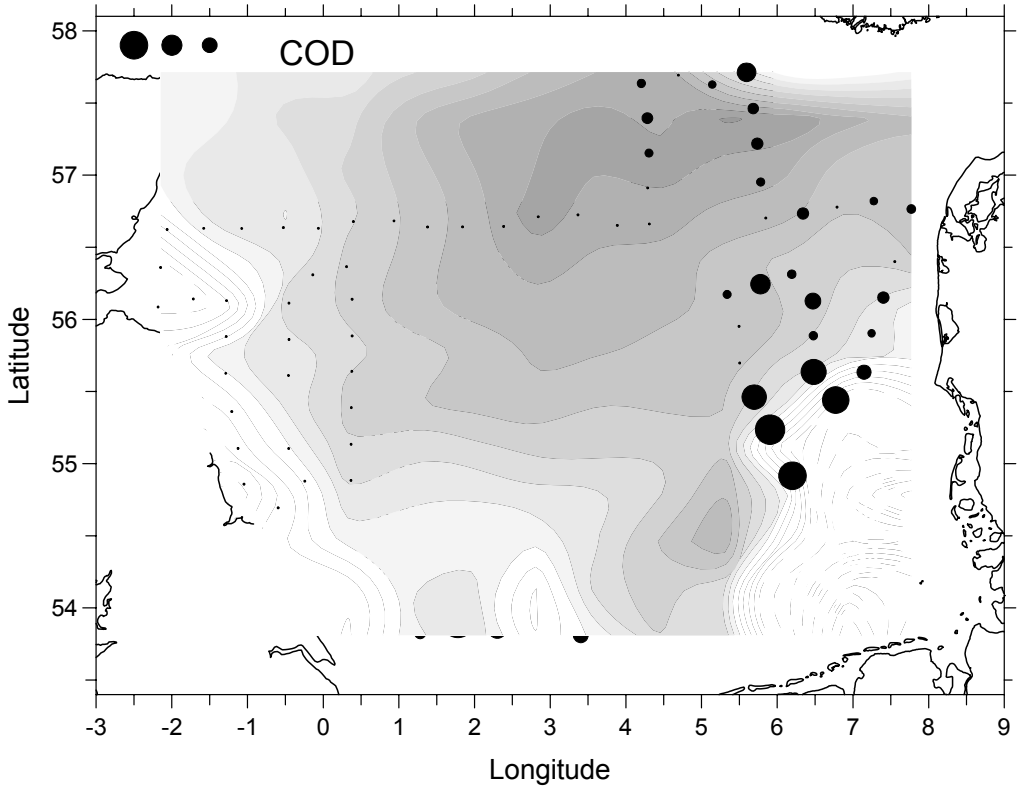

Figure 2.2.1 Larval abundance estimates of cod in no m^{-2} superimposed onto the contouring of surface water density kg m^{-3}, the three symbol circles (upper left corner) represent abundances of 2, 1 and 0.5 larvae m^{-2} respectively, data from March 1997 (Munk et al. 2002)

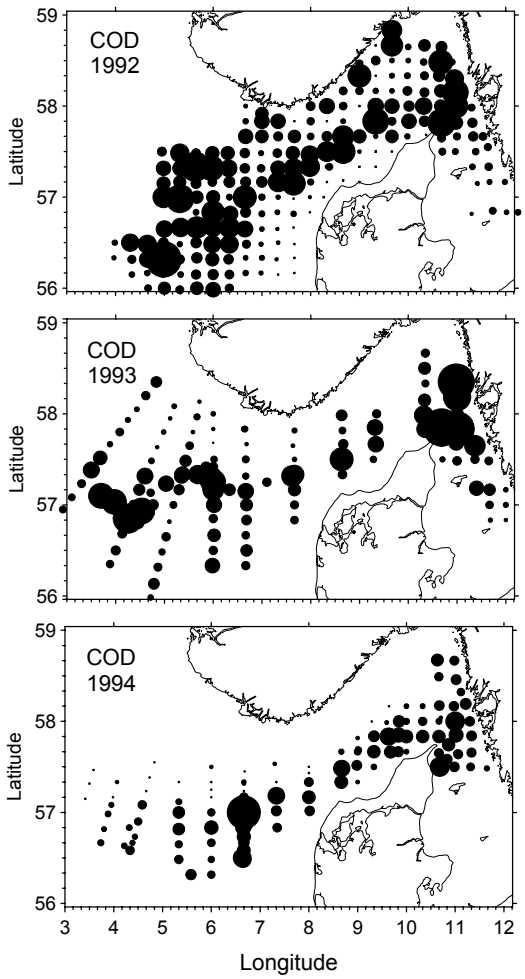

Fig 2.2.2 Distribution of cod larvae from 1991-1994, circles represent larval abundance in no m^{-2}, area of symbols proportional to larval abundance, maximum value of 3 m^{-2} (Munk et al. 1999)

As well as eggs, Thompson (1981) provide temperature dependent development rates for early larval stages of cod. Based on extensive studies in other areas and on experience from aquaculture it seems that first feeding larvae consume diatoms, dinoflagellates and tintinnids before moving onto the nauplii and copepodites of calanoid copepods, particularly those of *Pseudocalanus* (Last 1980, Economou 1991, Meeren 1991, Fossum & Ellertsen 1994, McLaren & Avendano 1995, McLaren et al. 1997). Prey size preference studies and a comparison between distribution of cod larvae and potential zooplankton prey show that the early life of cod depends strongly on prey availability in the right size range (Munk 1997).

2.3 Settlement and juveniles

It is known that cod probably require cryptic habitat into which to settle and that this may be a mechanism to avoid predators (Gregory & Anderson 1997, Bromley & Kell 1999). It is therefore possible that successful settlement could be limited to relatively small areas of suitable habitat (figure 2.3.1).

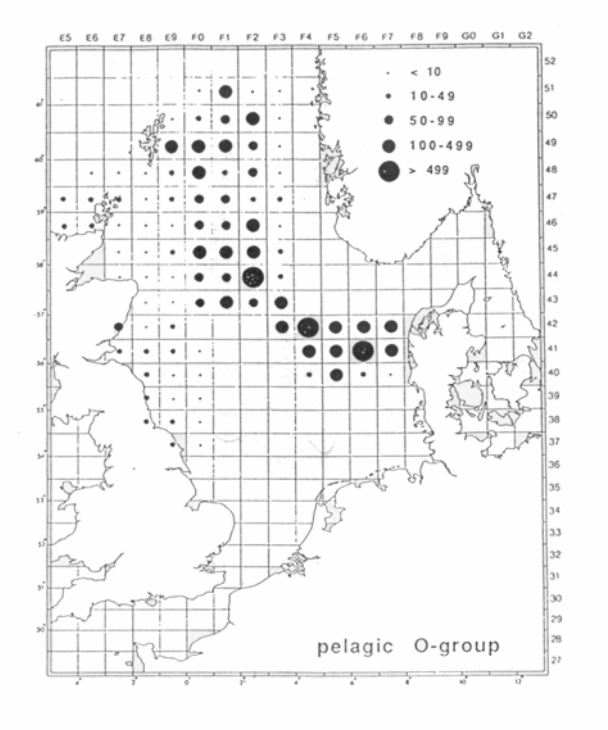

Figure 2.3.1. Average catches of pelagic 0 group cod averaged over June-July 1974-1983 (Brander 1994a).

Following settlement from the pelagic phase in June-August there is a shore-ward movement of 0 group cod (Riley & Parnell 1984). By October the fish are found in the tidal mudflats of estuaries of the English, Dutch and German coasts. The authors suggested this movement was due to the young cod seeking areas of reduced salinity. According to Daan (1978), the main nursery grounds in the period 1965-1974 lay along the Danish-German coast with a band of lower concentrations extending over the Dogger Bank and through the central North Sea. A similar picture was produced by Heessen (1993) for the period 1983-1987 (figure 2.3.2). Neither author had data on the distribution of settled young cod in the period before 1960 when the overall stock size was probably much lower than in the late 1960s.

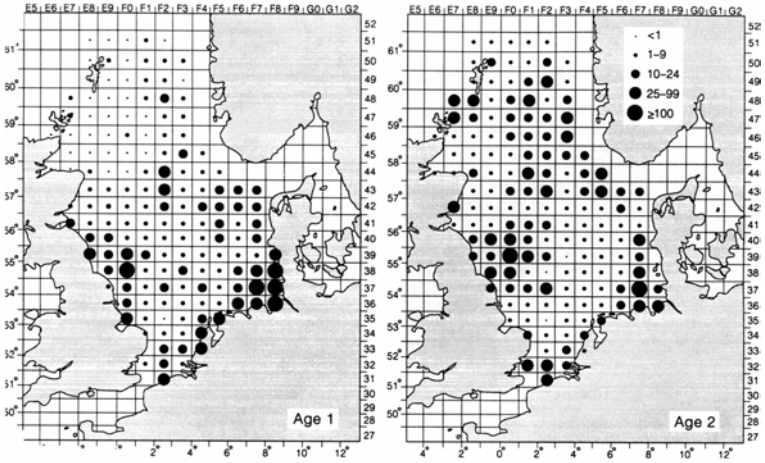

Figure 2.3.2. Distribution of cod, age groups 1 and 2 as mean number per hour fishing, averaged for period 1983 – 1987 (data from International Young Fish Survey (Heessen 1993)

More recent results from fisheries surveys appear similar although there has probably been a reduction in the abundance of 1 group fish along the Dutch coasts and German Bight (Figure 2.3.3a to 2.3.3c).

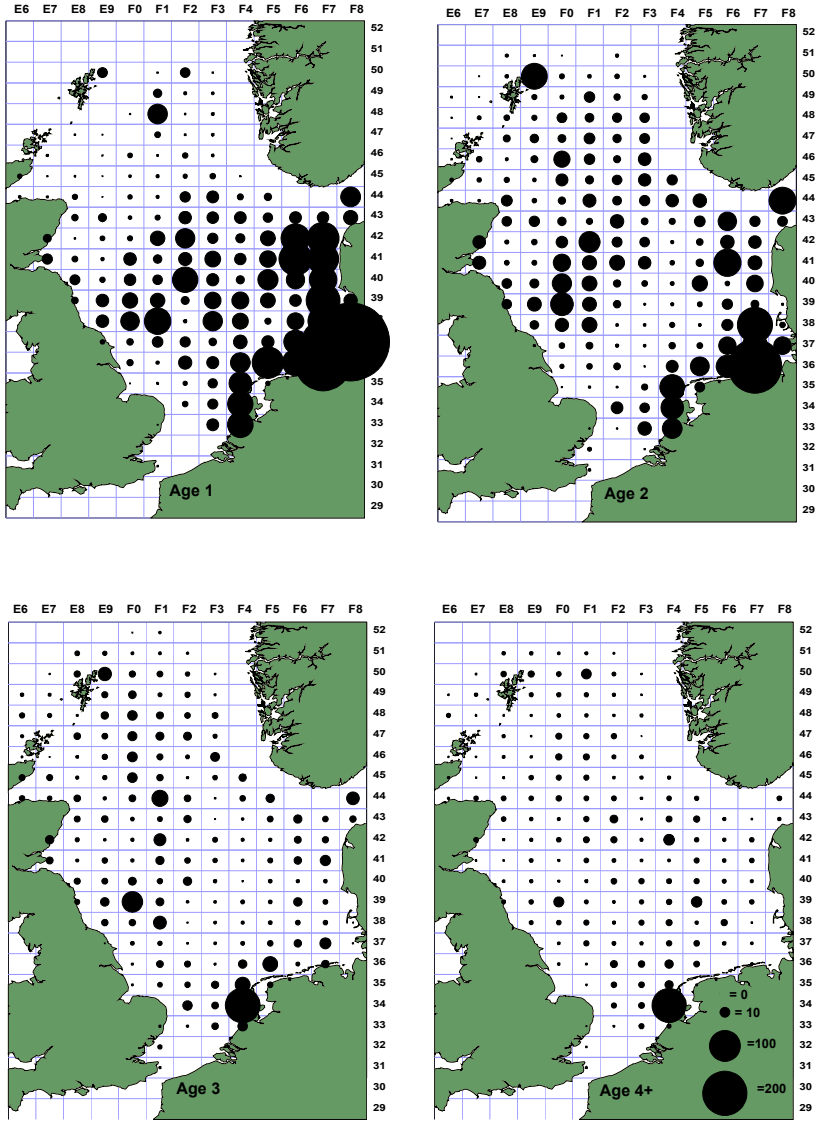

Figure 2.3.3a Average catches of cod by age from quarter 3 International Bottom trawl survey in period 1971-1981 (unpublished maps, A Tidd, CEFAS)

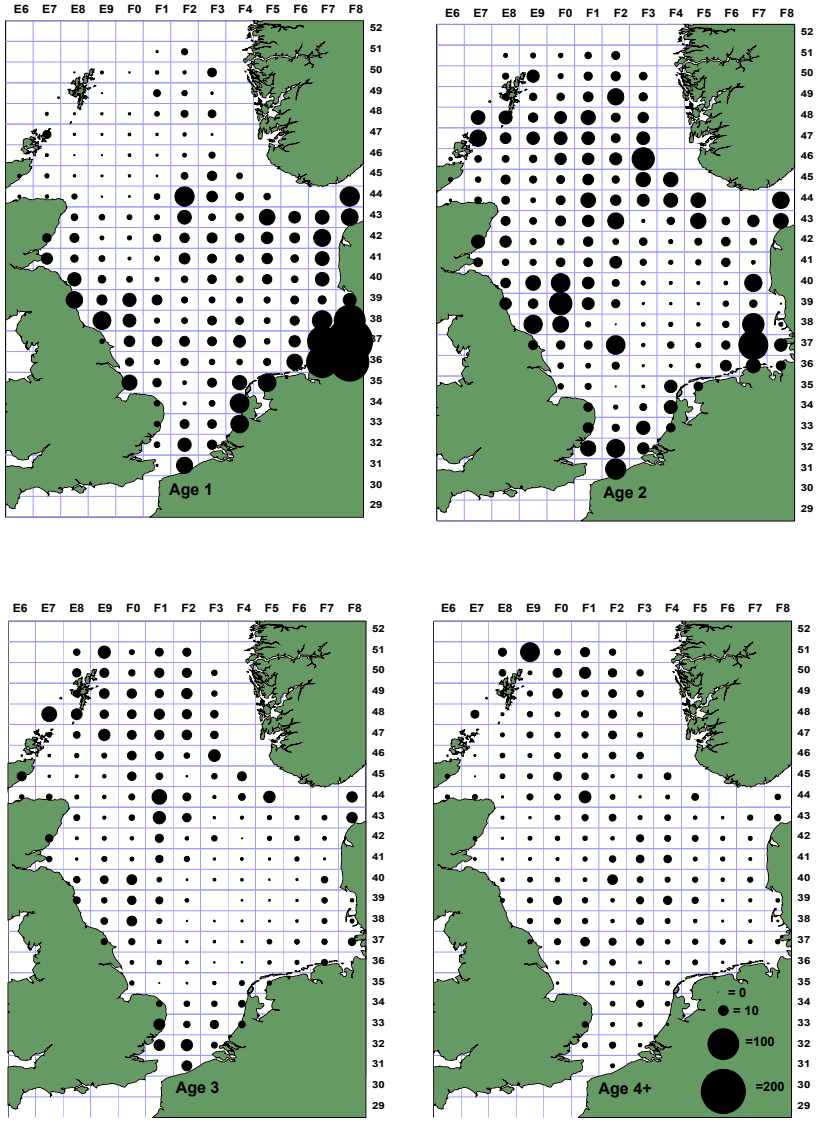

Figure 2.3.3b Average catches of cod by age from quarter 3 International Bottom trawl survey in period 1982-1992 (unpublished maps, A Tidd, CEFAS)

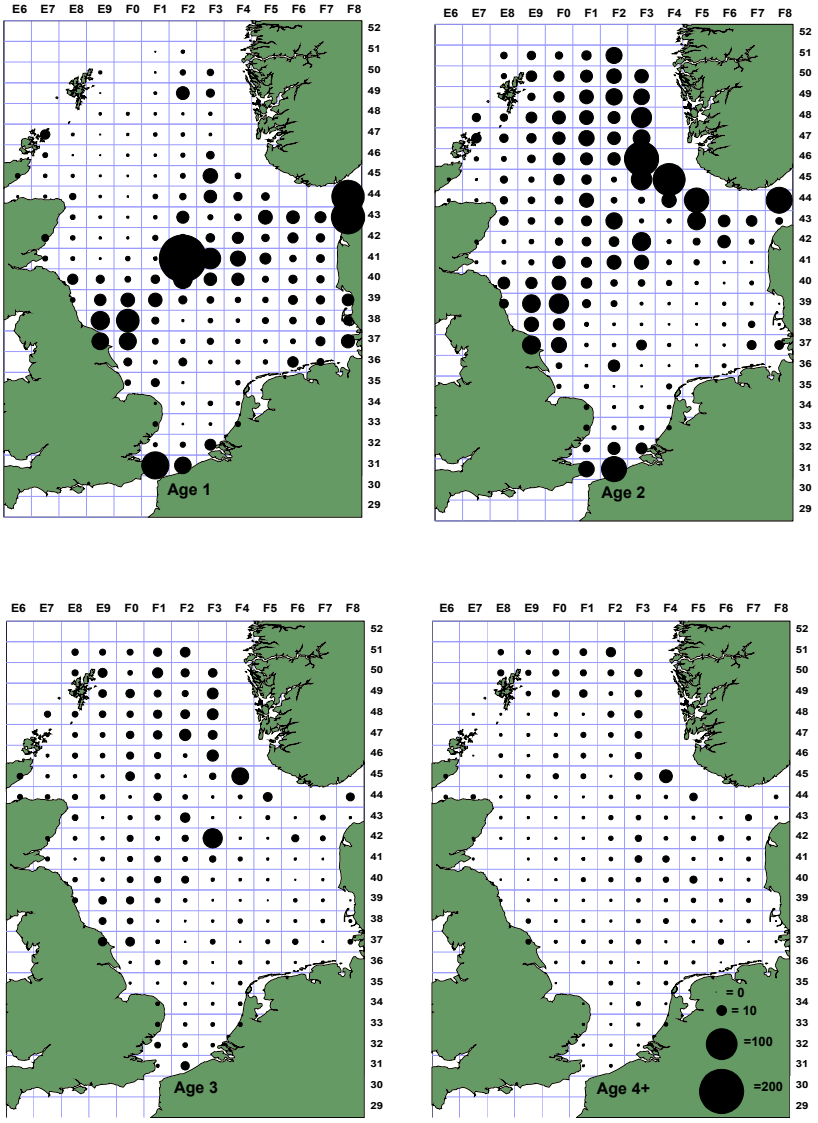

Figure 2.3.3c Average catches of cod by age from quarter 3 International Bottom trawl survey in period 1991-2001 (unpublished maps, A Tidd, CEFAS)

By the time the fish reach 3 years old they have moved off into deeper water and appear to be in a more constant thermal regime (Heessen & Daan 1994). Such observations accord with recent work on temperature experience of cod as determined using oxygen isotopes (Weidman & Millner 2000).

2.4 Recruitment

Average recruitment of North Sea cod has been in decline during the last few decades. As well as the effect of reduced reproductive potential, links have been proposed to changes in various environmental factors such as decline in abundance of *Calanus finmarchicus* (Sundby 2000). In addition, links have been made between increased sea temperatures and reduced recruitment for North Sea cod (Dickson & Brander 1993, Planque & Frédou 1999, O'Brien & Fox 2000). However, this still remains an intriguing statistical observation (which could be spurious) and research into mechanisms is required before such information can be utilised in management. Towards understanding controls on recruitment of North Sea cod, studies into the spawning locations, patterns of egg and larval drift, environment, prey and predation fields experienced during the early life stages would all be of value.

3 OVERVIEW OF CURRENT KNOWLEDGE OF PLAICE SPAWNING AND EARLY LIFE HISTORY

3.1 Plaice spawning

There have been numerous studies of plaice egg distributions since the beginning of the 20th century (Buchanan-Wollaston 1923, Simpson 1959, Harding et al. 1978, Harding & Nichols 1987, Heessen & Rijnsdorp 1989, van der Land et al. 1990). These show that plaice eggs are distributed widely throughout the English Channel and the southern and central North Sea. High egg concentrations have been observed in a number of locations, which presumably mark the centres of spawning activity. The regions of spawning are generally confined within the 50-meter depth contour (Harding et al. 1978). Major spawning centres were found in the eastern English Channel, the Southern Bight, the central North Sea and the German Bight. Other less important local spawning centres were found in the western English Channel and off the UK coast from Flamborough Head northwards to Moray Firth (Houghton & Harding 1976, Harding & Nichols 1987).

Buchanan-Wollaston (1923) was the first to develop techniques for estimating plaice egg densities and production, and implemented these in the Southern Bight (figure 3.1.1). Intermittent egg surveys were carried out between 1913 and 1971, and the knowledge of spawning locations was reviewed by Simpson in 1959, and by Harding (1978) (figure 3.1.2). The most recent plaice egg surveys in the North Sea were carried out between 1987-1989 and figure 3.1.3 shows the changing egg distribution observed during three cruises in 1989 (Heessen & Rijnsdorp 1989, van der Land et al. 1990). These illustrations (figures 3.1.1 – 3.1.3) essentially imply that the position of the spawning areas have changed little over the last century. Maturity levels in plaice sampled from the English fishery also suggest that even in the most recent years, the timing of spawning was similar to that recorded in the early years of the 20th century (Bromley 2000).

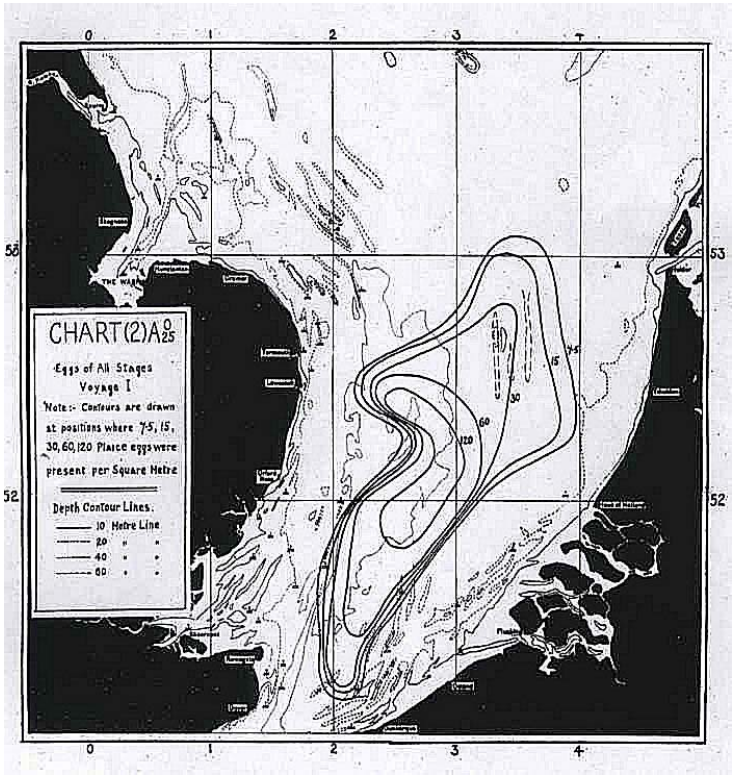

Figure 3.1.1. The distribution of plaice eggs (all stages) in the Southern Bight in 1913-14 (Buchanan-Wollaston 1923).

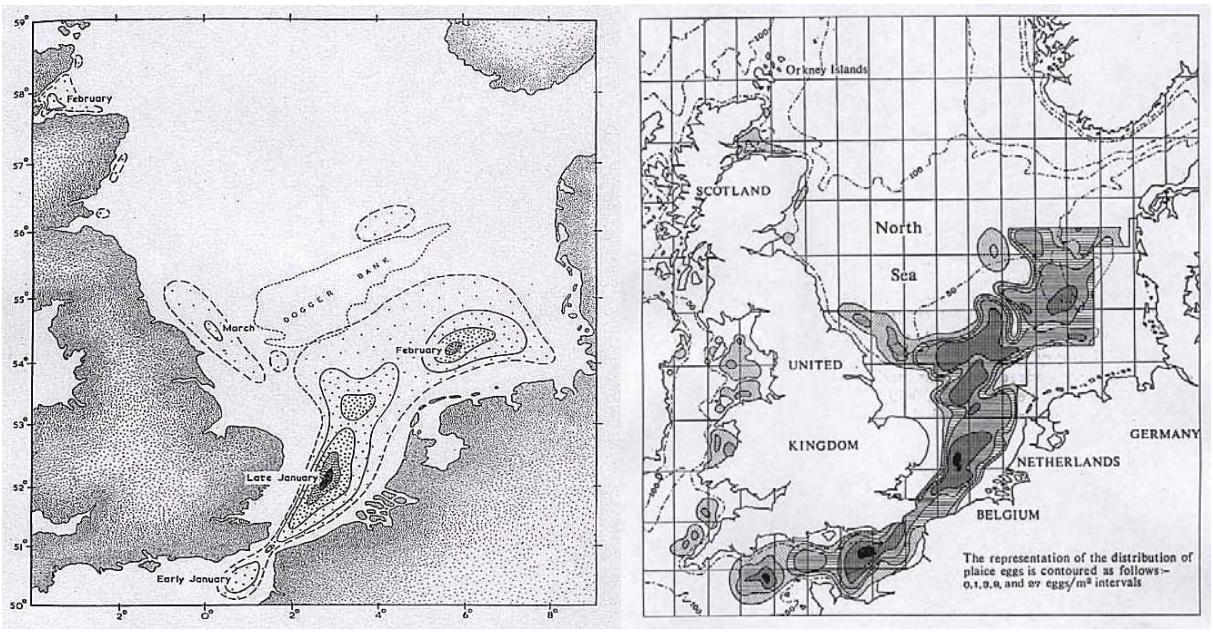

Figure 3.1.2. The spawning grounds of plaice illustrated by the distribution of stage I eggs. Left panel - A compilation of egg survey data collected between 1913 and 1952 (Simpson 1959).,Right panel - A compilation of egg survey data collected between between 1913 and 1971 (Harding et al. 1978)

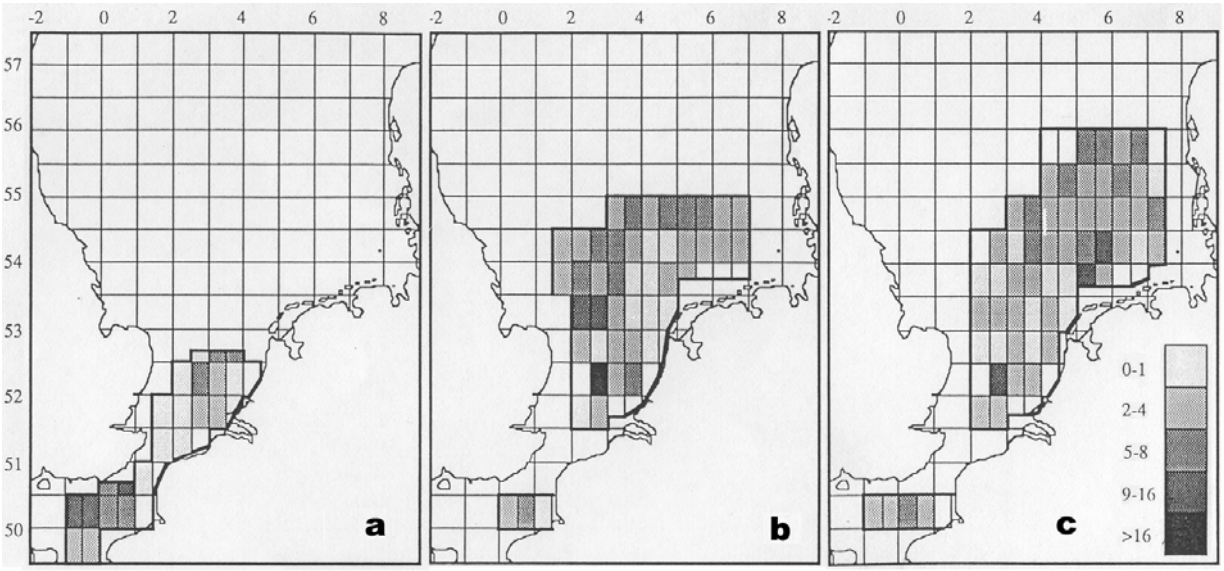

Figure 3.1.3. The distribution of stage I plaice eggs as observed during 3 surveys in 1989, a = 6-12 January, b = 10-20 January, c = 13 February-3 March (van der Land et al. 1990).

Spawning commences in December in the eastern English Channel and is progressively later moving northwards through the North Sea. Peak spawning occurs in late January in the Southern Bight, and in February-March in the more northern regions (Simpson 1959, Cushing 1969, Bromley 2000). The increased proportion of younger females in the spawning population, due to increased exploitation rates, may cause a delay and contraction of the spawning season (Rijnsdorp 1989). However, although the timing and duration of spawning can vary annually by ± 20 days, no clear trend through time was observed (Rijnsdorp & Vethaak 1997).

A clear diurnal rhythm in the spawning of plaice was observed in the Southern Bight, with most spawning occurring at night and only sporadic spawning during daylight (Simpson 1971, Nichols 1989).

Although the general pattern of spatial and temporal distribution of plaice spawning appears to change little between years, egg production studies have provided evidence that the relative importance of particular grounds as spawning locations may have changed through time. (Bannister et al. 1973) reported increased spawning activity in the German Bight and central North Sea compared to former years. Furthermore inter-annual fluctuations in total egg production have been observed on a regional (Harding et al. 1978) and North Sea scale (van der Land et al. 1990). The latter study estimated the total egg production in the Southern Bight in 1987-1989 at $1.8\text{-}2.6*10^{12}$, which is approximately half of the egg production ($5*10^{12}$) estimated by Buchanan-Wollaston (1923) for the years 1913-14. These fluctuations presumably reflect variations in spawning stock biomass, however changes in fecundity have also been observed (Horwood et al. 1986, Rijnsdorp 1991). Finally mortality rates differ between years and appear to be related to temperature. Low temperatures are associated with low egg mortality rates (Harding et al. 1978, van der Land et al. 1990). It is important therefore to have an updated view of the current situation of North Sea plaice spawning.

Development rates of plaice eggs are largely determined by water temperature (Ryland & Nichols 1975). Plaice eggs are passive and their dispersal is dependent on hydrodynamic forces and the physical properties of the eggs and ambient water (Talbot 1977, Coombs et al. 1990, Sundby 1991). Water movements by which plaice eggs are transported as passive particles determine the horizontal distribution of plaice eggs. Developing eggs and larvae from the Southern Bight spawning grounds drift on residual currents parallel to the coastline (Talbot 1977). The vertical distribution of eggs presumably will affect the horizontal distribution through exposure to variations in current speed and direction at different levels in the water column. The vertical distribution is firstly determined by the specific gravity of the eggs which is slightly positively buoyant. But plankton sampling in the southern North Sea showed eggs at all stages of development to be distributed throughout the water column, despite their positive buoyancy. This implies that tidally and wind induced mixing can strongly affect the vertical distribution of eggs (Coombs et al. 1990). Under calm weather conditions Pommeranz (1973), cited in Coombs (1990) reported plaice egg aggregations towards the surface.

3.2 Plaice larval distribution and development

The spatial separation of spawning areas (see above) and distribution of juvenile plaice (see below) implies that the developing eggs and larvae drift from the spawning grounds to the nursery areas. Although many studies have addressed the spawning and nursery areas of plaice, relatively few have focussed on processes during the pelagic egg and larval stages. The year-class strength in North Sea plaice appears to be determined during this pelagic phase but the mechanisms involved are unclear (Bannister et al. 1973, van der Veer 1986). Cold winters often produce strong year-classes and it has been suggested that cold temperatures may reduce egg and larval mortality by influencing predator activity or by synchronisation with the production of food organisms. Water temperature is also a reflection of the general weather system and associated atmospheric circulation patterns. Atmospheric conditions could in turn affect water circulation patterns and thus impact transport success to the inshore nurseries (van der Veer et al. 1998).

The swimming speeds of plaice at all larval stages do not enable them to resist horizontal displacement by currents. Therefore plaice larvae are often assumed to be passively transported by water movements (Talbot 1977, van der Veer et al. 1998). However, vertical migration behaviour may affect the results of transport by residual currents and this behaviour has been described in several species of flatfish larvae ((Koutsikopoulos et al. 1991, Campos 1996). Plaice larvae move to the seabed during metamorphosis, while they are still offshore outside of the nursery areas (Harding & Talbot 1973). Cushing (1975) suggested that the bottom dwelling larvae are passively transported towards the inshore nursery areas by residual bottom currents. Creutzberg (1978) and Rijnsdorp (1985) reported that the late larval stages of plaice entering the estuarine nurseries of the Dutch coast show vertical migrations synchronised to the tidal cycle, resulting in a net onshore movement (figure 3.2.1). This selective tidal stream transport has also been reported as mechanism for larval transport in other species and for adult migrations in plaice and cod (Harden Jones et al. 1979, Boehlert & Mundy 1988, Hill 1991, Metcalfe & Arnold 1997, Righton et al. 2001).

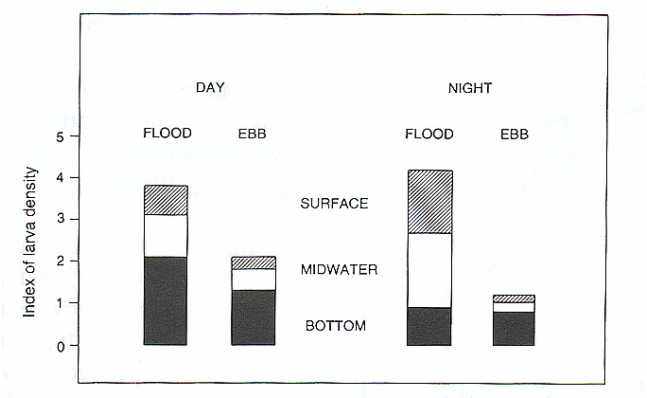

Figure 3.2.1. Catches of plaice larvae in the Eastern Scheldt at different levels in the water column, during the day and night, and during flood and ebb tide (Rijnsdorp et al. 1985)

3.3 Settlement and juveniles

In plaice the nursery grounds are spatially segregated from the feeding and spawning areas of the adults. Juveniles inhabit sandy and muddy habitats in shallow coastal and estuarine waters along all of the coastlines of the English Channel and the southern and central North Sea (Edwards & Steele 1968, Zijlstra 1972, Riley et al. 1981, Riley et al. 1986, Van Beek et al. 1989, Rogers et al. 1998). The 0-group mainly occupies the intertidal regions and plaice exhibit a progressive offshore movement with increased fish length (figure 3.3.1).

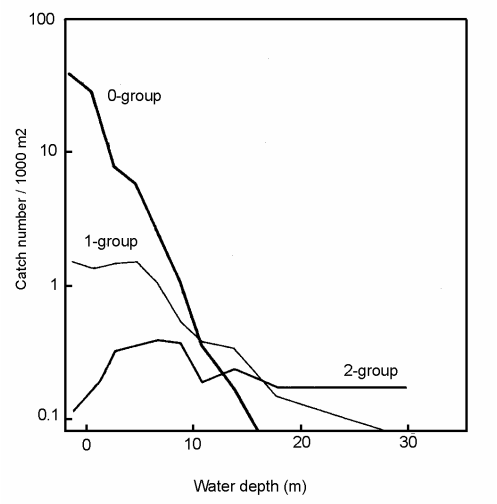

Figure 3.3.1. Catch rates (number/1000m^2) of 0-, 1- and 2-group plaice in relation to water depth (modified from Riley (1981)

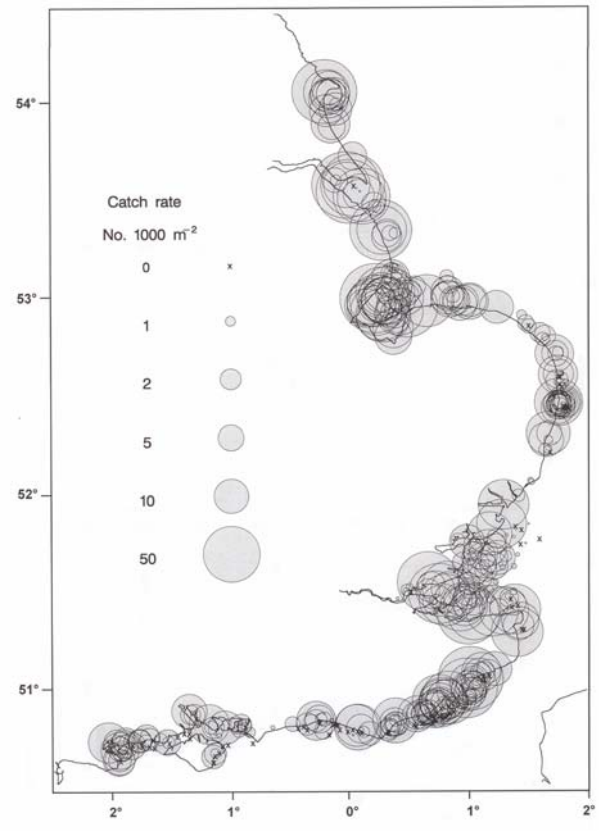

Figure 3.3.2. Mean annual catch rates (numbers/1000m^2) of 0-group plaice from 1981 to 1997 for each station of the English Young Fish Survey (Rogers et al. 1998).

Figure 3.3.2 shows the distribution of 0-group plaice along the English coasts of the North Sea and eastern English Channel. In figure 3.3.3, the average catch rates per sector along the English coasts have been scaled to the catch rates per sector along the continental coasts (by using relative gear efficiency estimates). This demonstrates that the English nursery areas are probably relatively unimportant considering the North Sea as a whole The relative importance of certain sectors as nursery grounds also varies inter-annually (Van Beek et al. 1989) but the most important nursery areas for North Sea plaice are the German Bight and the estuarine Wadden Sea (figure 3.3.4). These nurseries contribute the majority (50-90%) of the recruits to the North Sea plaice stock. (Zijlstra, 1972, Van Beek *et al.*, 1989, Anon., 2001). Growth and survival conditions are favourable in the Wadden Sea, due to large food resources and the relative scarcity of predators. Optimal growth rates and relatively low mortality rates have been observed for 0-group plaice in the Wadden Sea (Zijlstra et al. 1982, Bergman et al. 1988, van der Veer et al. 1990, Beverton & Iles 1992, van der Veer & Witte 1993).

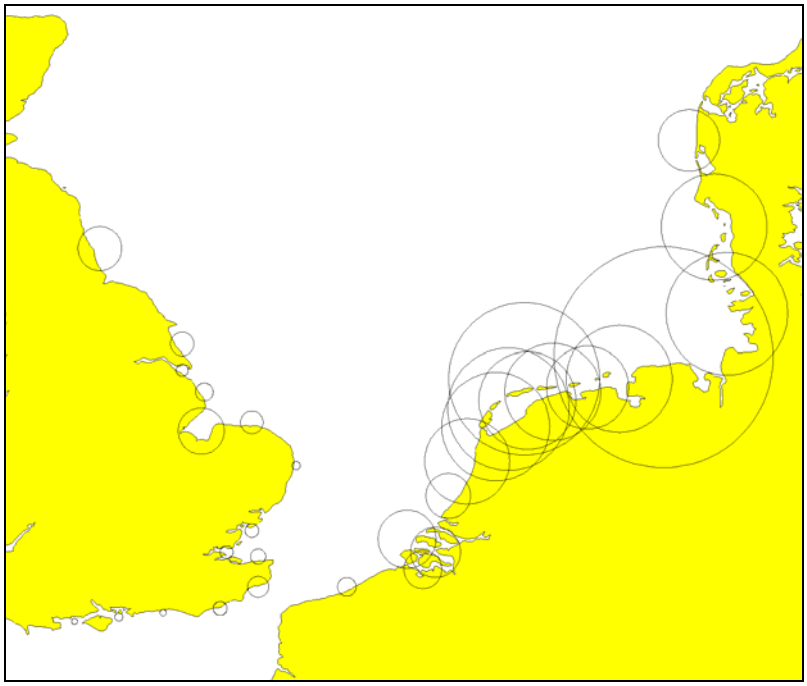

Figure 3.3.3. Density indices of 0-group plaice based on the mean annual catch rates (numbers/1000m^2) by sector of the English Young Fish Survey, the Dutch Demersal Fish Survey and the Dutch Sole Net Survey. All data are from the period 1981 to 1997. The catch rates have been corrected for relative gear efficiency estimates [Anon, 2001 #2946]

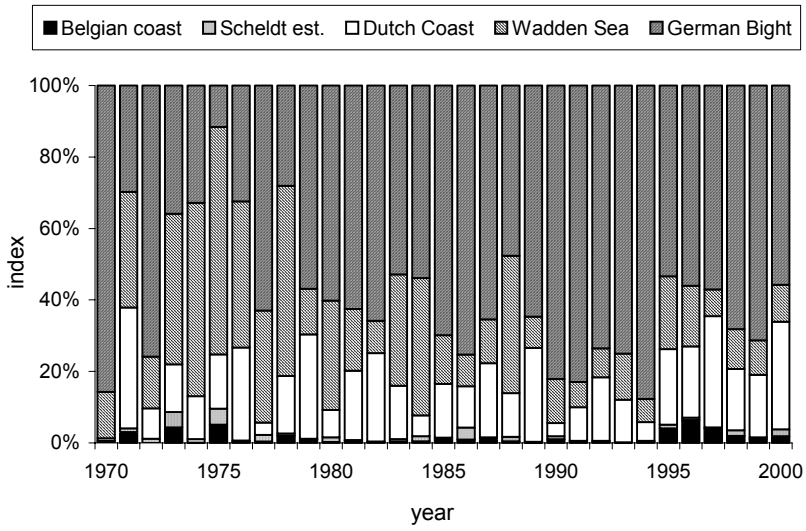

Figure 3.3.4. Annual indices for the relative production of 0-group plaice by sector for the Dutch Demersal Fish Survey. Production is estimated by multiplying the mean catch rates (numbers/1000m^2) per sector with the surface area of the sector. Unpublished data continuing the time series presented by Van Beek (1989).

3.4 Recruitment

As with cod, links have been made between reduced sea temperatures and improved recruitment for North Sea plaice (Bannister et al. 1973, van der Veer & Witte 1999, Fox et al. 2000). However, this still remains an intriguing statistical observation and research into mechanisms is required before such information can be utilised in management. Possible mechanisms include effects upon survival during the transport from spawning to nursery grounds and predation on settled plaice by Crangon on the nursery grounds. Further studies into the patterns of egg and larval drift, environmental, prey and predation fields experienced during the pre-recruit stages would all be of value

4 RECENT UN-PUBLISHED SURVEY DATA FROM 2003

A survey for ichthyoplankton was performed in the North Sea during 5-19 March 2003, with the Norwegian research vessel "Haakon Mosby". The coverage was carried out between 54-59^{0}N and 121 stations were taken. On each station the water masses were profiled with CTD, and vertical sampling was performed with WP-2 nets from the bottom to the surface. All fish larvae were sorted out of the samples, measured and identified onboard the ship, while the rest of the samples were fixed in formaldehyde and the fish egg sorted out in the laboratory. Eggs were identified to species based on size (egg diameter), oil globules, colour or pigmentation of the embryo. If eggs could not be identified they were put into 0.2 mm size groups (e.g. 1.0-1.19, 1.2- 1.39, 1.4-1.59 mm).

Very few cod eggs were found during the survey. The few eggs that were positively identified as cod were found in low concentrations in scattered areas as shown in figure 4.1.

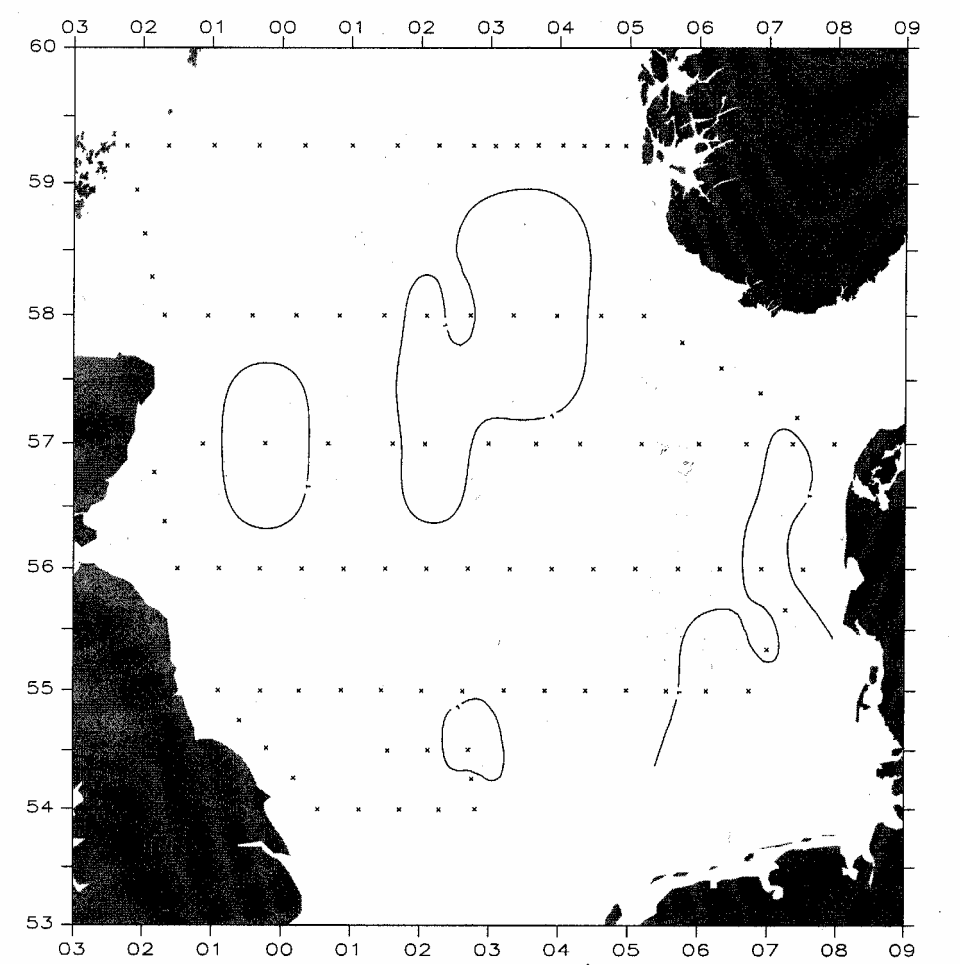

Figure 4.1. Distribution of cod eggs in numbers per m^2 observed during a survey with RV " Haakon Mosby", 05-19 March 2003.

Many more haddock eggs were found, especially in the northern part of the area with peak concentrations along the Scottish coast (figure 4.2).

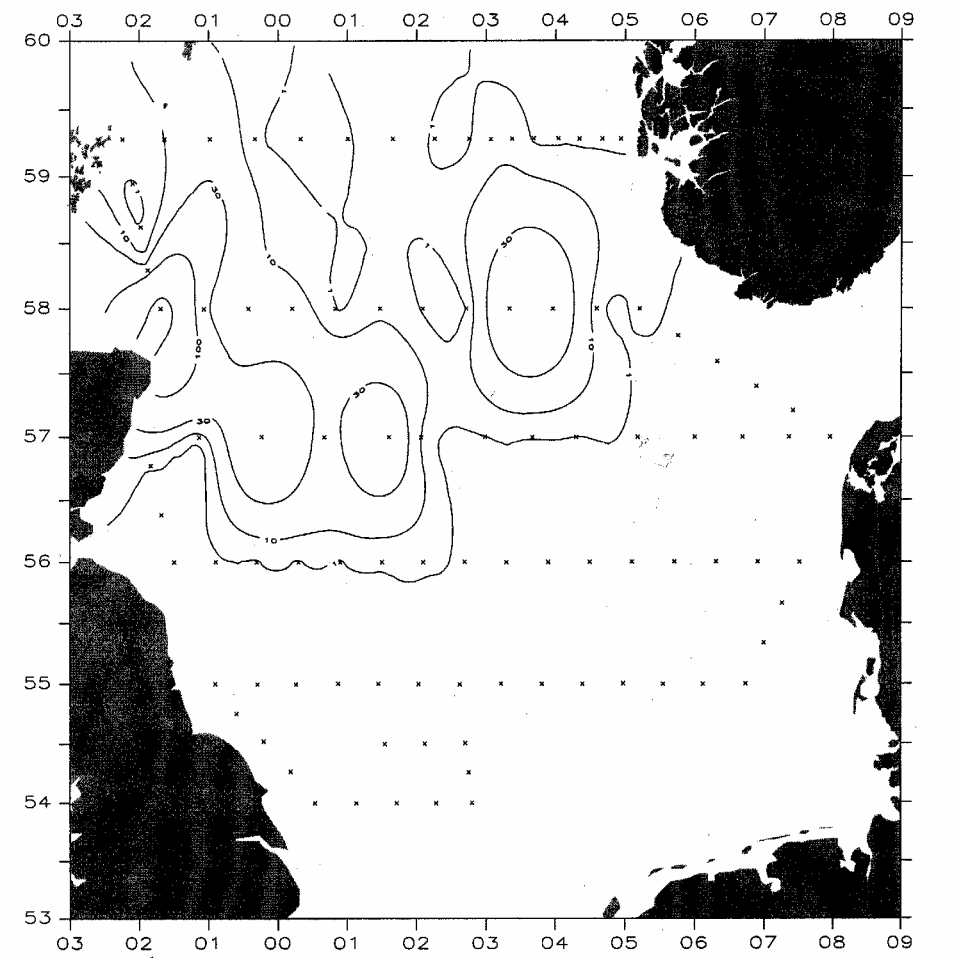

Figure 4.2. Distribution of haddock eggs in numbers per m^2 observed during a survey with RV " Haakon Mosby", 05-19 March 2003.

Many unidentified eggs in size group 1.4-1.59 mm were found in the same area and most of them were probably haddock (figure 4.3).

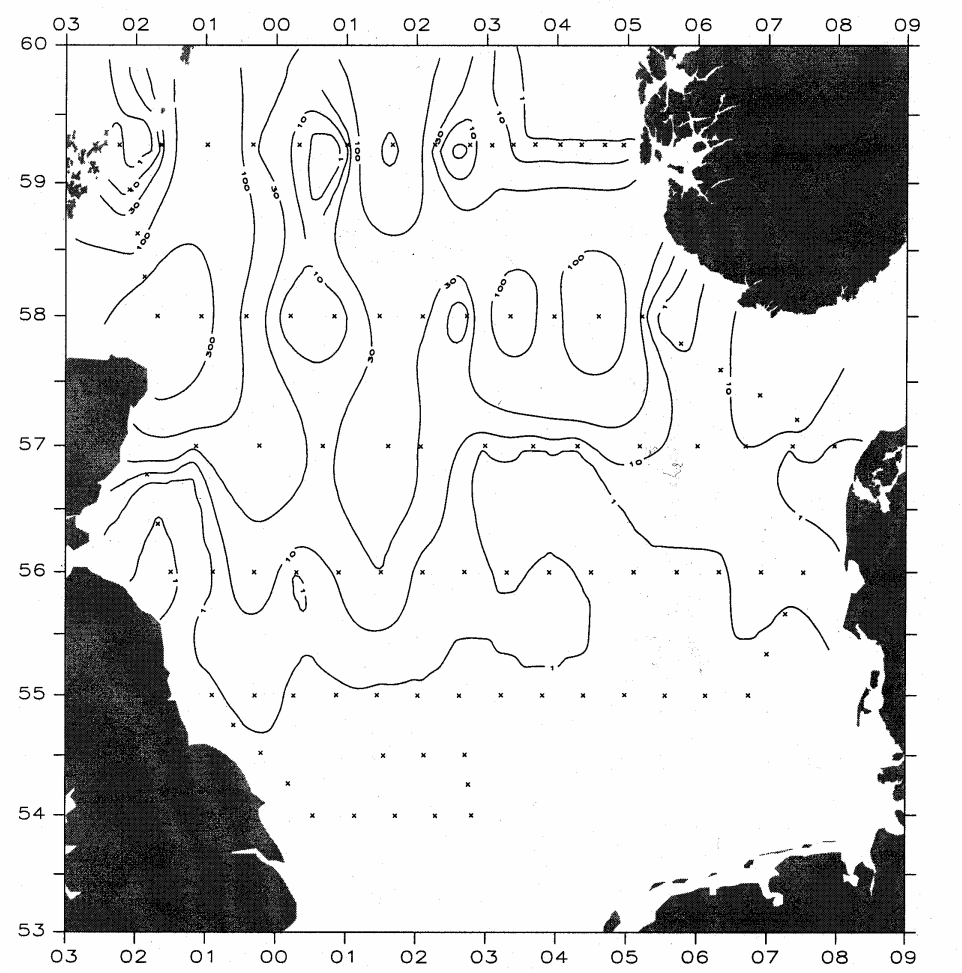

Figure 4.3. Distribution of cod-like eggs in the size group 1.4-1.59 mm in numbers per m^2 observed during a survey with RV " Haakon Mosby", 05-19 March 2003.

There were only a few plaice eggs found in the surveyed region. They were found in scattered concentrations along the Danish coast, in the central part of the North Sea and along the north-east English and east Scottish coasts (figure 4.4).

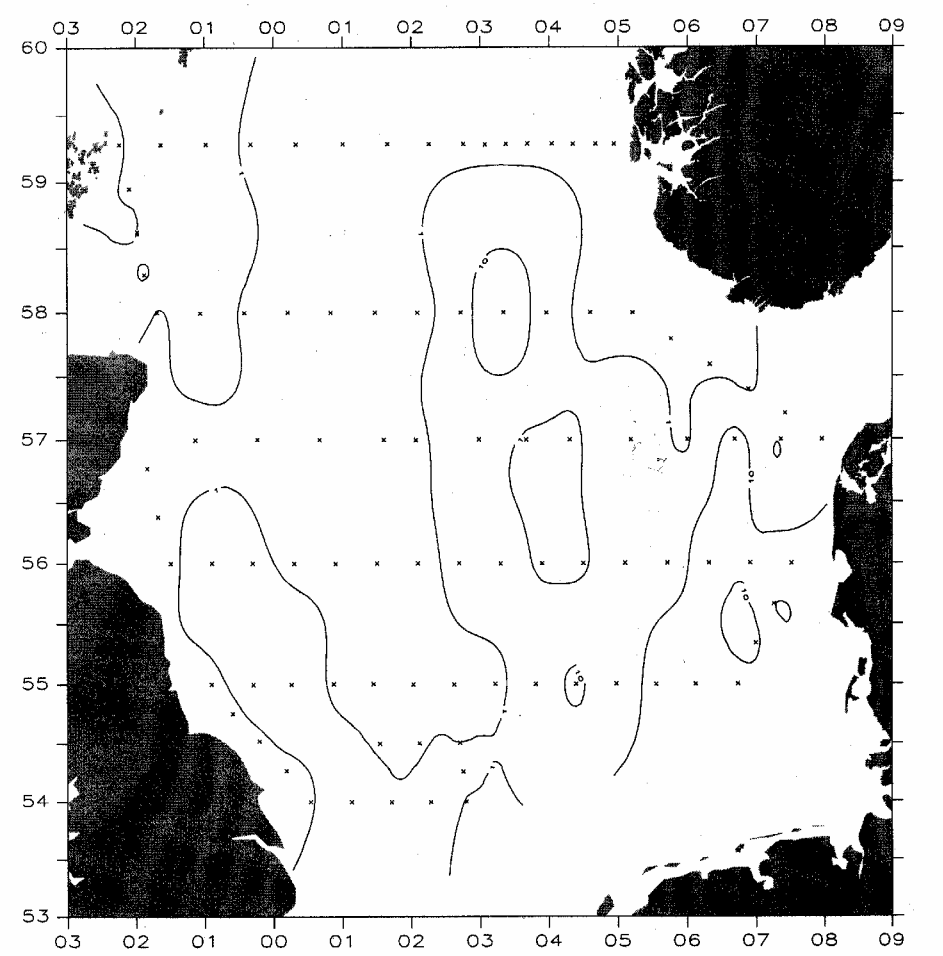

Figure 4.4. Distribution of plaice eggs in numbers per m^2 observed during a survey with RV " Haakon Mosby", 05-19 March 2003.

5 REPORT ON PROGRESS WITH ICHTHYOPLANKTON SURVEYS UNDERTAKEN IN THE IRISH SEA AND DEVELOPMENT OF GENETIC PROBES.

The unambiguous identification of fish eggs of several species remains problematic. Of particular relevance to North Sea studies are cod, haddock and whiting whose eggs are all of similar appearance and over-lap in size range. Only when the eggs have reached developmental stage V can they be confidently identified based upon developing patterns of pigmentation in the embryos. Since the majority of eggs sampled in ichthyoplankton surveys are at younger developmental stages, this limitation has posed severe problems with previous surveys designed to identify the spawning ground of these species (Brander & Hurley 1992, Heath et al. 1994). During surveys in the Irish Sea in 1995 and 2000, iso-electric focussing was used to try and overcome this problem (Mork et al. 1983, Armstrong 1997, Armstrong 2002). Unfortunately success was limited and only around 50% of the samples analysed produced positive identifications. Furthermore the technique requires pre-sorting eggs and analyzing them at sea or freezing them at –80^{o}C at sea for subsequent analysis. Whilst pre-sorting is possible it takes time and it was felt that the low rates of success might have been caused by protein degradation prior to freezing. Also storing large numbers of frozen eggs at –80^{o}C sea is difficult and requires the use of liquid nitrogen dewars or portable ultra-low temperature freezers. In response to this Defra have funded the development of genetic based methods for egg identification. The aims of this development project were to

- produce a system capable of identifying eggs of any developmental stage of cod, haddock and whiting
- to automate such a system so that it can rapidly handle hundreds to thousands of samples
- to recommend sampling protocols to be used at sea for collecting samples for subsequent genetic identification

A semi-automated method has been developed involving robotic extraction of DNA from individual eggs stored in ethanol or DMSO and subsequent analysis on an automated PCR real-time detection system (Perkin-Elmer, TaqMan). The system is capable of identifying whether eggs are those of cod, haddock or whiting with greater than 95% accuracy for all developmental stages (Taylor et al. 2001).

The original hope was that this system would prove reliable using eggs fixed with formaldehyde. This would be an ideal solution since genetic analysis could then be bolted on the back of standard laboratory pre-sorting, only those eggs identified as cod-like would need to be gene probed. Unfortunately we have not been able to get the system to work reliably with formaldehyde fixed material. It is known that formaldehyde degrades DNA so this result was not entirely unexpected although several workers have reported successful PCR from formaldehyde fixed samples. We have investigated the effects of using other preservatives such as ethanol and DMSO for fish eggs. Whilst these do not degrade DNA they do cause shrinkage and cause eggs to become opaque. These effects invalidate the use of standard size keys for identifying the eggs and would prevent egg-staging, a necessary step in estimating egg age.

In March 2003, an ichthyoplankton survey was undertaken in the Irish Sea in which cod-like eggs were pre-sorted at sea and individually preserved in ethanol. Due to the relatively calm weather conditions this was successfully accomplished. The samples are currently being genetically analyzed at the University of East Anglia using the TaqMan system. Several hundred eggs have already been analyzed and DNA quality appears high. Cod and haddock eggs are producing positive results but there are some problems with whiting eggs that are not reacting as expected. This may indicate that the current batch of probe for the whiting species needs to be re-synthesized by Perkin-Elmer. Unfortunately a full set of results were not available in time for PGEGGS. These results will be presented at the planned autumn PGEGGS/PLACES meeting.

In addition, work on methods that may work with formaldehyde fixed material is continuing under the EU project MARINEGGS. However, it is unclear whether reliable protocols will be available in time for the 2004 surveys. PGEGGS should therefore plan on the necessity to pre-sort cod-like eggs at sea preserving them in ethanol if genetic analyses are to be applied.

6 IMPLICATIONS OF EXISTING KNOWLEDGE FOR THE DESIGN OF EGG SURVEYS IN 2004

Sections 2-5 provide detailed information on the known spawning areas for cod and plaice and techniques for egg identification. From this information and acknowledging available resources, PGEGGS have drawn the following conclusions regarding design of an ichthyoplankton survey for these species in the North Sea.

6.1 Overall aims

PGEGGS propose extensive egg surveys for cod and plaice to be carried out in the North Sea during winter and spring 2004 by a partnership of Dutch, Danish, German, UK and Norwegian institutes. The surveys will be undertaken to:

a) Investigate all areas of the North Sea for the distribution of cod and plaice eggs
b) Identify and delimit areas with high concentrations of cod and plaice eggs
c) Trace the sites of intensive spawning based on distributional information of egg stages and larval sizes.
d) Correlate the distributional patterns of eggs and larvae to hydrographic features, and investigate potential physical/biological linkages
e) Assess the change in distribution of identified egg/larvae concentrations between separate surveys
f) Describe the distribution pattern of eggs/larvae of non-target species

6.2 Sampling design

In the southern North Sea, spawning of cod and plaice occurs between December and March. Spawning occurs progressively later moving northwards so that egg production continues until April in the central and northern areas.

Sampling should commence in the Channel and the Southern Bight in January and the second part of February. After covering the southern North Sea in January, the total North Sea south of 62^0N will be covered once in early March.

The horizontal distribution of eggs is dependent on stationary and transient factors that respectively are conserving and changing the distribution patterns of the fish eggs. The result can be complex patterns of distribution that need dense sampling to be resolved. On the other hand the ship time and the need for a synoptic coverage will reduce the station density which is practicable in a large-scale survey.

Due to this necessity to balance spatial station density and available resources (section 8) with the requirement to cover a large sea area, PGEGGS have decided that a minimum of two hauls shall be taken per ICES rectangle.

7 PLANNED AND EXISTING SURVEYS WHICH WILL CONTRIBUTE TO CO-ORDINATED NORTH SEA ICHTHYOPLANKTON SURVEYS

PGEGGS main task is to co-ordinate existing and planned ichthyoplankton cruises, initially in 2004, to undertake a comprehensive survey for cod and plaice spawning in the North Sea (adopted acronym PLACES). In this section we summarise the available ship time and describe existing activities.

7.1 International Herring Larvae Surveys in the North Sea

International Herring Larvae Survey take place in the North Sea at given time periods and survey units. Beside others, three surveys are scheduled annually in the Southern North Sea in the second half of December (conducted by The Netherlands), first (Germany) and second half of January (The Netherlands). The station grid is shown in figure 7.1.1. A spatial and temporal overlap with plaice and cods spawning activity is likely to appear.

In the herring larvae program modified GULF III samplers are used (Dutch Torpedo and German Nackthai). These high-speed samplers are operated in a double oblique manner at 5 knots ships speed. Net opening is 20 cm in diameter and mesh size is 300 µm. Stations are 10 nm apart and each ICES rectangle is covered with 9 stations. Samples are preserved in 4% buffered formalin/freshwater and stored for a minimum of 48h fixation-time prior to sorting. This procedure would prohibit the pre-sorting of "cod-like" eggs for genetic analysis, but will be sufficient for plaice egg identification. In addition, data on abundance of cod-like eggs even without positive speciation would be valuable.

Available information from the IHLS program is that fish eggs are caught but only partly sorted. The total number of all fish eggs per survey varies between years, but is something between 2000 and several thousand in total.

Numbers of fish larvae caught per station excluding herring is low, and from these most abundant are flatfish and *Ammodytes* larvae.

During the German surveys in January 2004 there is the opportunity to add an extra sampling program for cod and plaice eggs after the completion of the herring survey. Available ship time for such a program is 3 to 4 days (16.01. – 20.01.04, see section 8).

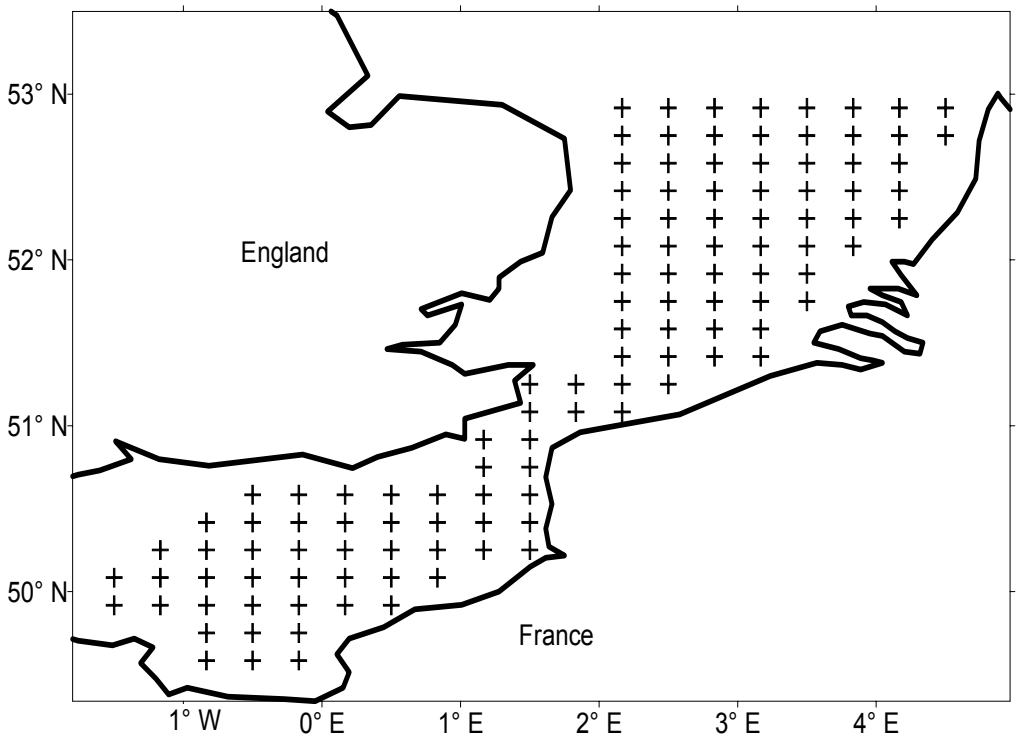

Figure 7.1.1: Stationgrid during the herring larvae surveys in the Southern North Sea.

7.2 Embryonic malformation survey

The German "Bundesforschungsanstalt für Fischerei" in Hamburg has a program on malformations of embryonic fish larvae running from 24.02. – 09.03.04. The BFA will participate in the planned survey activity and offer additional ichthyoplankton sampling during a cruise with RV Walther Herwig from 24.02. – 09.03.04 (figure 7.2.1). This will cover the German Bight and parts of the Southern Bight.

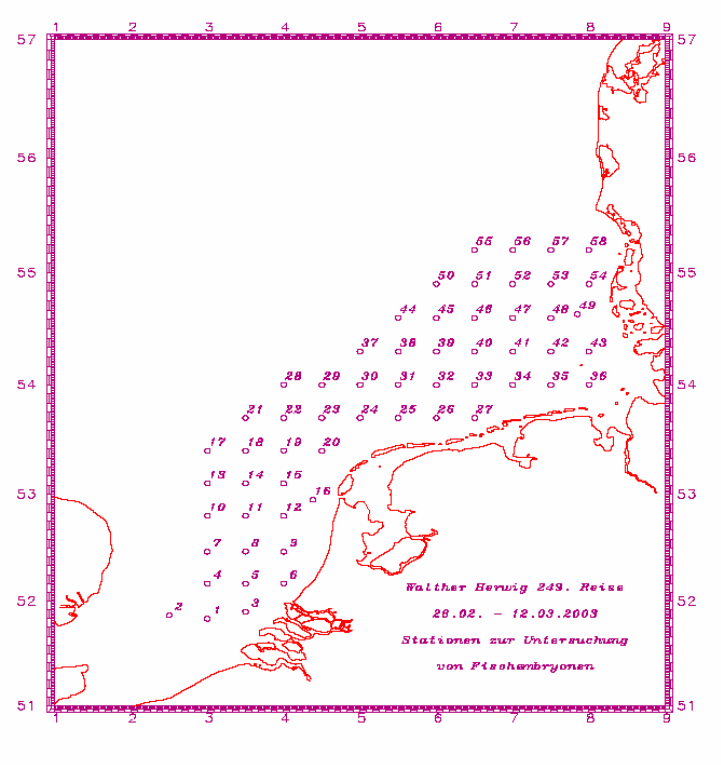

Figure 7.2.1: Station grid for the examination of malformations in embryonic fish larvae during the 249th cruise of RV Walther Herwig. Will be the same during the cruise from 24.02. – 09.03.04.

7.3 Short summary of a planned sampling programe for 2004 in the framework of the German GLOBEC project

As a contribution to the international cod and plaice survey in the North Sea, the German Globec project "Trophic interactions between fish and zooplankton under the influence of physical processes" could provide a back up with an intensive field sampling campaign being carried out in 2004. Sampling within this program will cover all trophic levels from phytoplankton to planktivourous fish and will focus on the seasonal dynamics under the influence of pysical forcing. Sampling will cover 56 stations in the German and Southern Bight on a regularly spaced grid with approx. 18 nm grid point distance (figure 7.3.1).

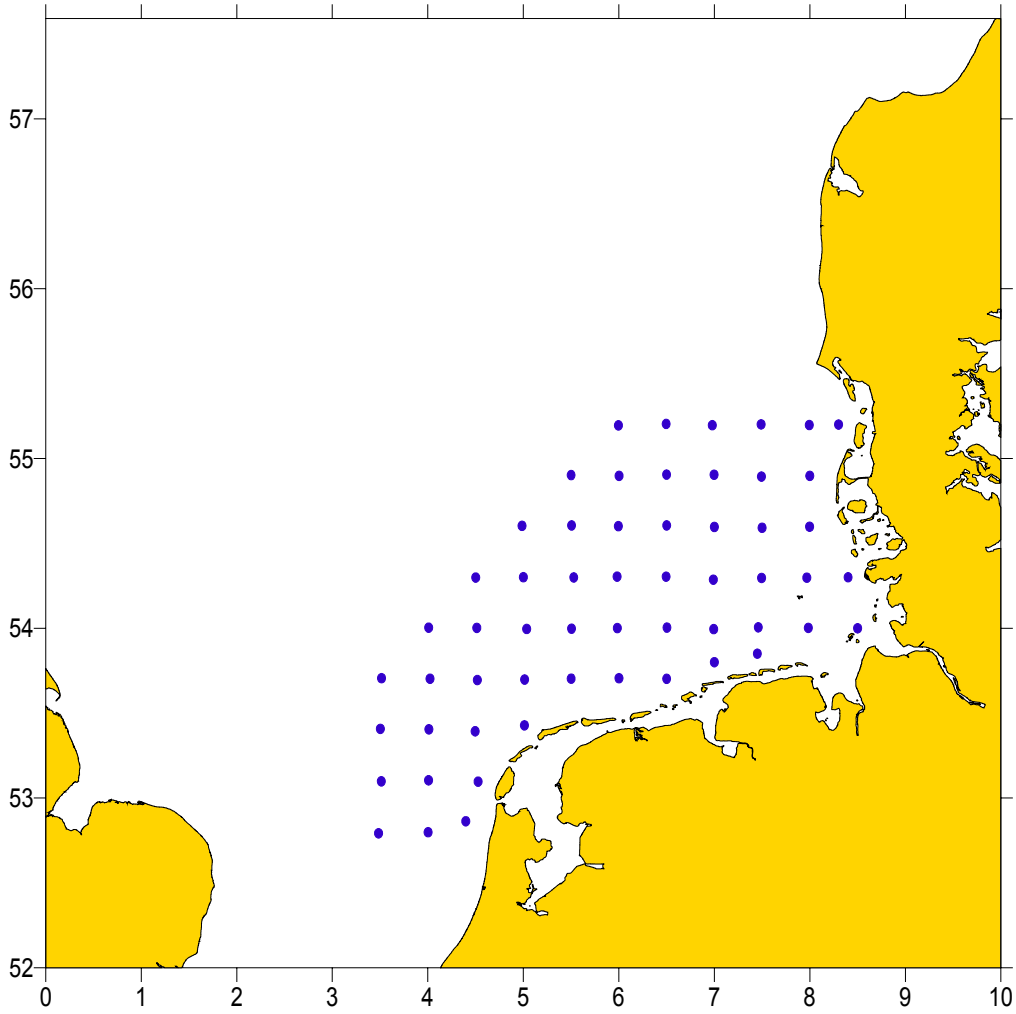

Figure 7.3.1. Planned sampling grid for the German Globec programm in the Norrth Sea in 2004 (56 stations, 18 nm grid point distance).

Cruises will be carried out at a frequency of approx. 3-4 weeks starting in mid February and to mid October. Peak spawning time of cod in the German Bight is around day 60, while peak spawning of plaice in this area may be two to four weeks earlier. The first GLOBEC cruise starting 16 Feb, 2004 will be suitable for sampling cod and plaice spawning in the German/Southern Bight. For this cruise approx. three weeks of ship time are planned, which may enable two coverages of the grid.

Standard gear applied on each station will be CTD/O probe, Bongo nets equipped with 500, 300 and 150 µm mesh size, as well as an Apstein net (50µm). In addition, a multifluoroescence probe will be applied to profile chlorophyll. On focus stations, which are still to be defined, standard sampling will be complemented by a number of additional measurements covering, e.g., nutrients, zooplankton production, vertical distribution and patchiness of ichthyo- and zooplankton as well as stock structure and distribution of adult fish. For a gear list see table 7.3.1

Table. 7.3.1. Gear list German GLOBEC program.

Type	Mesh/Measured	Deployment
Scanfish	P, T, S	Undulating
CTD/O	P, T, S, O, L	Vertical
Multifluor.	Chlorophyll	Vertical
Bongo	500; 330; 150	Double obl.
WP2	150	Vertical
Multinet		
0.25 m²	150	Vertical
0.5 m²	330	Vertical/Obl.
Apstein net	50	Vertical
LHPR	200; 50	Undulating
IPR	200/Video	Undulating
VPR	Video	Undulating
Combi-Trawl	10mm	Pelagic/Demersal
Hydro-acoustics		

7.4 Time offered by other institutes for PLACES

CEFAS offer 21 days on RV 'Corystes' from Feb 17 to 9 March.

Netherlands Institute of Fisheries Research will apply for 10 days seatime on RV 'Tridens' and for 1 week onboard of a commercial vessel.

DIFRES expects to be able to reserve 10 days seatime on RV 'Dana' from 1 March.

IMR expects to be able to reserve 14 days seatime on RV 'H.Mosby' or RV 'J.Hjort' in March.

8 PROPOSED WORK PROGRAM

In this section we describe our proposed work program for PLACES 2004

8.1 Sampling strategy

The group has decided on the general outline of the sampling strategy. When funding and requested shipping time have been allocated the group will reconvene to confirm the sampling strategy.

8.1.1 Spatial coverage

PLACES will cover the entire North Sea and the eastern English Channel. The survey area has been divided into 7 regions, based on the coverage of ongoing research programmes and on the expected North South gradient in spawning activity (figure 8.1).

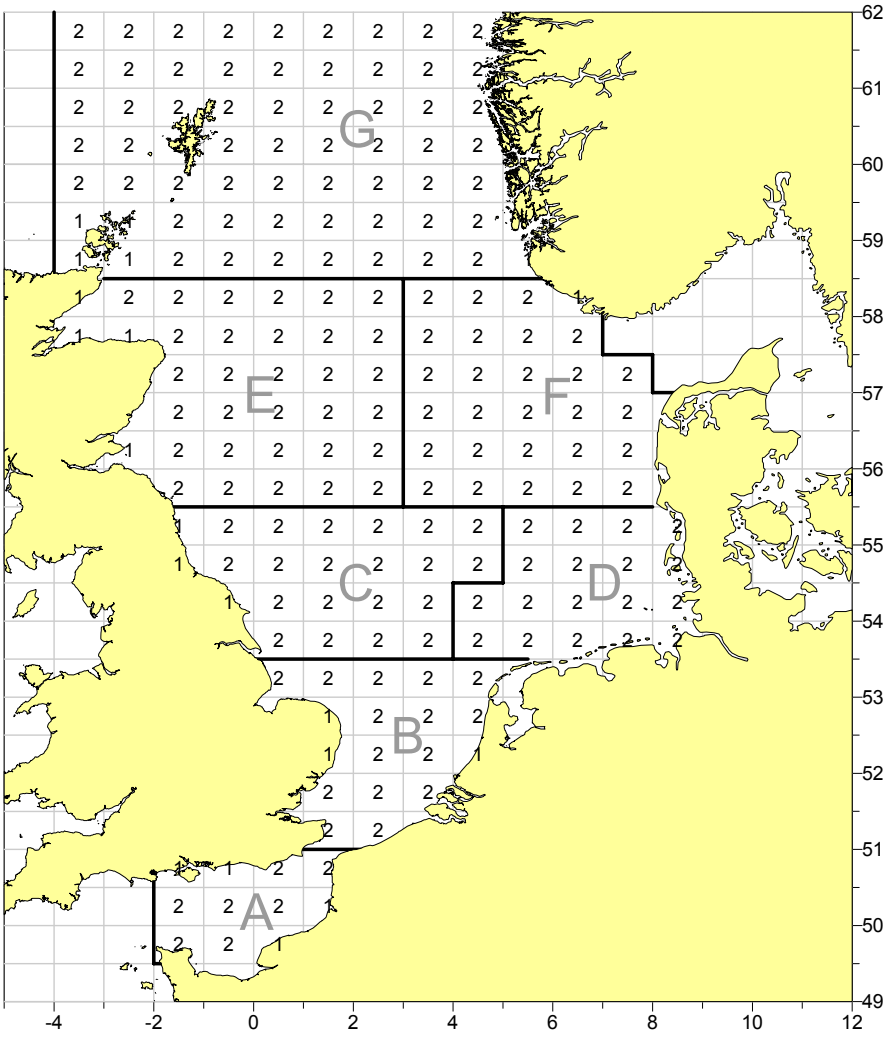

Figure 8.1. Map of the survey area indicating the regions (coded by letters) and the number of samples per ICES rectangle.

8.1.2 Sampling grid

In order to obtain a large spatial coverage the sampling grid is set at two samples per ICES rectangle and at one in some coastal rectangles (figure 8.1). In principle this means one sample per ½ ICES rectangle (east-west division). However when samples are obtained from ongoing research programmes the grid may deviate within the limitations of at least two samples per ICES rectangle. This results in a total of minimum 374 stations (table 8.1).

Table 8.1. Overview of the number of ICES rectangles and stations within the survey area and an estimation of the minimum days at sea required to sample all stations at least once.

Region		Number of ICES rect.	Number of stations	Days required for single coverage*
Area A	VIId	11	18	2
Area B	51'00" - 53'30"	18	33	4
Area C	53'30" - 55'30" west	22	43	5
Area D	53'30" - 55'30" east	19	36	4
Area E	55'30" - 58'30" west	35	66	7
Area F	55'30" - 58'30" east	28	55	6
Area G	58'30" - 62'00"	64	123	13
Total area	VIId + IV	197	374	41

** approximately 10 samples per day*

8.1.3 Temporal coverage

The major goal of PLACES is to map the spawning distribution of plaice and cod. At a minimum only a single survey coincident with peak spawning is required. However, as the spawning period is progressively later from south to north and there is some uncertainty in when peak spawning will occur, the egg surveys should cover more than one period. Table 8.2 indicates when ship time is available and when the cruises are planned (or requested). The highest priority is to cover each region at least once. If weather conditions are favourable the surveys can be expanded by extending the area covered by each cruise or by increasing the numbers of samples per ICES rectangles. No final decision has yet been made on this issue.

Table 8.2. Overview of the planned / requested shipping time. The hatched cells indicate when a vessel is not available. The number of days available/requested specifically for PLACES is indicated and for each cruise the sampling regions (refer to figure 8.1) are shown in parentheses.

week	Germany Alkor	Germany W.Herwig	England Corystes	Norway H.Mosby	Denmark Dana	Netherlands Tridens	Netherlands Charter
51						IHLS (A+B)	
52							
1							
2	IHLS (A+B)						
3	4 days (A)					5 days (B)	
4						IHLS (A+B)	
5							
6							5 days (B-D)
7							
8	GLOBEC (B+D)		7 days (C)				
9	GLOBEC (B+D)	MAL. (B+D)	7 days (E)				
10	GLOBEC (B+D)	MAL. (B+D)	7 days (E)	7 days (G)	7 days (F)	5 days (B+C)	
11				7 days (G)	3 days (F)		
12							
13							
14	GLOBEC (B+D)						
15	GLOBEC (B+D)						
16	GLOBEC (B+D)						

8.2 Staffing for cruises

A minimum of six people is required onboard of the research vessels due to the pre-sorting of cod-like eggs for genetic analyses (see section 8.4) and need to work watches in order to sample 24 h per day. At least two members of the scientific crew must be well trained in sorting out eggs. As no research vessels are available from 26 January until 16 February we will request funding to allow the charter of a vessel. Depending on the facilities of the charter vessel, egg pre-sorting may or may not be feasible.

8.3 Sampling at sea and analysis of samples

PGEGGS agreed to standardise plankton sampling gear and protocols as feasible. Formaldehyde fixed plankton samples will be analyzed using standardised protocols by the participating institutes. Analysis of samples by commercial sorting centres will also be considered. This is more fully described in section 9.

8.4 Genetic analysis of eggs

Because of the problems experienced with performing genetic analyses on formadehdye preserved samples, we recommend that cod-like eggs are pre-sorted at sea after each plankton haul and the eggs preserved in eppendorfs filled with ethanol. Such pre-sorting has been successfully undertaken during the Irish Sea ichthyoplankton surveys run in 1995 and 2000 but does however become increasingly difficult in poor weather conditions. Samples for genetic analysis will likely be analyzed at University of East Anglia, U.K. providing an application for funding for this to UK Defra is successful.

8.5 Hydrographic measurements

The group agreed that plankton sampling should be accompanied by hydrographic measurements in order to describe environmental patterns favouring high concentrations of fish eggs and larvae. As a minimum requirement, calibrated measurements of temperature and salinity should be provided at each station. The measurements could be obtained from probes mounted to the plankton sampler. Measurements obtained from this type of sampling may suffer from comparatively slow response times of conductivity sensors. Vertical profiling from stationary ships are proposed as an

alternative resulting in precise hydrographic measurements. Further this will enable additional measurements such as fluorescence to be recorded and collection of water samples for e.g. nutrients and chlorophyll. To obtain measurements of standard quality, only regularly calibrated sensors should be used. Vertical profiling of hydrography will be carried out by DIFRES, IFM, BFA and IMR, CEFAS and Neth. Inst. Fish. Res. will explore the option to undertake additional vertical hydrographic measurements.

8.6 Additional measurements

CUFES (Continuous Underway Fish Egg Sampler) has been used to produce high-resolution maps of fish egg distributions. CUFES could be employed during PLACES surveys if the equipment were available. PGEGGS decided that CUFES should not be a high priority for 2004 but a pilot would be useful if resources became available (see section 9.11).

Vertical distribution of eggs can be studied with multi-nets or systems such as LHPR. PGEGGS agreed that individual institutes might wish to incorporate such work into PLACES but it will not form a part of the core research program.

Additional measurements are welcome within PLACES providing that the core program is retained.

9 AGREED PRELIMINARY PROTOCOLS FOR PLACES

PLACES will comprise a co-ordinated series of ichthyoplankton surveys in the North Sea in 2004. In order that the data from different institutes can be combined in a reliable manner, PGEGGS members agreed to adopt standard protocols for sampling, sample analysis, data handling and analysis. Below we present the broad overview of these protocols, the detaiuls will be developed during the autumn project co-ordination meeting.

9.1 Sampling gear

The standard plankton sampler for CEFAS will be a Gulf VII high-speed plankton sampler (Nash et al. 1998). This sampler has a 76cm diameter unencased body fitted with a 40cm diameter aperture, conical nosecone. The standard net will be made of 270μm aperture mesh.

The CEFAS sampler is fitted with a 'Guildline' conductivity, temperature, depth (CTD) sensor unit. This has been modified to relay 'real-time' flowmeter (and environmental) data back to a shipboard display unit. A Valeport BFM 001 type flowmeter (with a blade diameter of 12.5cm) will be centrally mounted inside the sampler nosecone with its boss 2.5cm back from the leading edge. Another BFM 001 flowmeter will be mounted externally on the sampler frame to provide an accurate measure of distance travelled (D) and sampler speed through the water. The ratio between internal and external flowmeters will provide an index of clogging (C).

The Netherlands Institute for Fisheries Research (former RIVO) may be able to borrow a GULF VII PRO-NET system (Spartel Ltd, Totnes,Devon) together with CTD and flow monitoring system. This system uses Valeport BFM002 type flowmeters (with a blade diameter of 5cm) which will be mounted in similar positions to the larger flowmeters mounted on the CEFAS sampler.

A theoretical volume filtered can be calculated on each tow by multiplying the area of the nosecone aperture (A) by the distance travelled by the sampler (D). This theoretical volume can then be compared with actual volume filtered (calculated from flowmeter readings) and a sampler efficiency calculated for each station.

Where clogging occurs (i.e. sampler efficiency falls below 70% of A x D or where the index C falls below 0.6), nets of 400μm aperture mesh will replace the standard 270μm net. If clogging continues to be a problem, then a reduction in nosecone size to a 30cm diameter aperture is advised.

The others Institutes involved may use other gears, e.g. GULF III type samplers or BONGO nets. Procedures should be standardized as much as possible.

The BONGO net is 60 cm in diameter and can be equipped with nets of different mesh sizes (typically ranging from 150 to 500 μ). Two samples are taken at each hauls in parallel. Both nets can be set up with flowmeters which shouldbe placed in the centre of the net-opening. When operating the BONGO net, additional CTD profiles should be made available by e.g. Rosettes.

The standard sampler will be deployed on a double oblique tow, at 3 knots, from the surface to within 2 metres of the bottom (or as near as bottom topography will allow) and return to the surface. The requirement is an even, 'V' shaped dive profile, filtering the same volume of water per unit of depth. The aim will be to shoot and haul at the same rate with the sampler spending 10 seconds in each 1 metre depth band. At shallow stations, multiple double-oblique dives may be necessary to enable a sufficient volume of water to be filtered. A minimum sampler deployment time of 15 minutes is recommended.

9.2 Sampling at sea

The standard plankton samples collected for PLACES will be analysed onshore and should be treated carefully when being fixed at sea. The procedure will be as follows:-

a). Remove the end bag used on the station before washing down the net.

b). Attach a clean end bag and **gently** wash down the net from both ends of the sampler, taking care to wash the lower surface of the net just in front of the end bucket.

c). Always wash down from the nosecone end last.

d). Make sure net is clean, using more than one end bag if necessary.

e). Make doubly sure that a clean end bag is left on the sampler ready for the next station.

f). Wash the plankton from the end bags into a jug and proceed with the presorting of cod-sized eggs.

9.3 Pre-sorting eggs for genetic analysis

Because of problems with analysing formaldehyde fixed material with geentic tools, participants in PLACES will need to pre-sort cod-like eggs at sea. The following procedure wil be adopted

a) After the catch has been recovered from the cod-end of the plankton sampler, then container (jug) containing the eggs should be stood on ice.
b) Remove small aliquots and remove suitable looking eggs to small drops of water placed in a petri-dish. During this step the petri-dish should be stood on ice to prevent degradation of the eggs.
c) When 10-15 eggs have been pre-sorted transfer the petri-dish to the low-power microscope. Determine egg diameter (if possible image analysis system such as PISCES (CEFAS, Lowestoft) will prove very useful.
d) Confirm the egg is cod-like (i.e. no oil globules, un-segmented yolk), measure the egg diameter (if using system like PICES, the required egg diameter range can be marked with chinagraph on the compyuter monitor making confirmation that the egg size is in the correct range very rapid), record the egg diameter, determine and record the egg developmental stage
e) Transfer the egg to a labelled eppendorf tube (use of an automated label printer such as Brady PC-TTL) will ensure tubes are clearly labelled and that label will resist ethanol.
f) Using a 50 ul Gilson pipette, remove any excess seawater from the eppendorf.
g) Add 1 ml of ethanol (Analar grade), seal tube, confirm egg is still in tube by holding eppendorf up to the light
h) Store eppendorfs in plastic trays at room temperature.
i) Repeat stages b – h until up to 75 eggs have been sorted on the station.
j) On completion, concentrate the remainder of the sample using a 270 um mesh sieve and transfer into the plankton storage jars adn fix using 4% formaldehyde.
k) Top up the jar with 4% formaldehyde, making sure that the volume of plankton does not exceed 50% of the volume of the jar.
l) Any excess sample should be fixed separately in additional jars.
m) Put labels containing station details in pencil into all jars, also marking cruise and station number on jar lid with chinagraph pencil will aid selection of plankton jars during laboratory sorting..

Caution: Be sure to keep separate labelled jugs and seives for formaldehyde and pre-formaldehyde use. It is important that the material for genetic analysis does not come into contact with formaldehyde.

It is vital that accurate records are kept as the informatiuon on egs pre-sorted at sea will have to be combined with the data from the laboratory sorting of the formaldehyde fixed portion. It is recommended that paper records are entered regularly into electronic form as a back-up.

Experience in the Irish Sea ichthyoplankton surveys conducted in 2000 suggests that pre-sorting up to 75 eggs will take around one hour. However, on most stations the number of suitable eggs in the sample will be much lower.

9.4 Sorting of samples in the laboratory

The standard fixative for use on these surveys will be a 4% solution of buffered (pH 7 - 8) formaldehyde in either distilled or fresh water. (for example, CEFAS use 250g of sodium acetate trihydrate is dissolved in 10 litres of 30% formaldehyde to make a buffered stock solution. The stock solution is then diluted to 4% using distilled water). This solution is approximately iso-osmotic with sea water and will minimise damage and distortion of the eggs.

9.4.1 Overview of procedure

It is recommended that the 4% formaldehyde is drained from the sample immediately before analysis. The sample can then be made up to a known volume using an odourless 'observation fluid'. This 'observation fluid' is comprised of 0.5% propylene phenoxetol, 4.5% propane-1, 2-diol and 95% distilled water, by volume. This solution will act as a preservative on fixed material and enables the sample to be sorted without toxic formalin fumes building up in the laboratory.

The identification of cod, haddock and possibly some smaller diameter plaice eggs can be difficult if all three species are spawning in the same area. Plaice eggs are generally much larger than those of other species spawning in the North Sea. Russell (1976) gives an egg diameter of 1.66-2.17mm. In addition, plaice eggs have a thicker membrane than either cod or haddock.

The main identification problem will be to distinguish between cod and haddock eggs. The egg diameter range is given by Russell (1976) as 1.16 - 1.89mm for cod eggs and 1.2 - 1.7mm for haddock. Neither egg has any distinct morphological features, which would aid identification. In the later stages of egg development the embryos develop characteristic larval pigmentation which enables separation of the two species. During PLACES, genetic methods will be employed to distinguish early stage cod and haddock eggs.

The eggs of cod and plaice will be classified into one of six morphological stages (IA, IB, II, III, IV, and V) following the development criteria described for cod (Thompson & Riley 1981) and plaice (Simpson 1959).

All eggs identified and staged should also have their diameter measured and recorded. All unidentified eggs (usually without distinguishing features such as oil globules) should also be measured. A minimum diameter for egg staging will be decided at the autumn project meeting.

9.4.2 Sub - sampling protocol

Where large numbers of eggs and larvae occur in plankton samples it becomes impractical to sort the total sample. The recommended method for sub-sampling is by using a folsom splitter. In this way, samples can be sub-divided repeatedly to achieve the optimum sampling level. It is recommended that 100 eggs of the target species (cod or plaice) are present in the sub-sample. If more than 100 eggs of these species are sorted from the sample (or sub-sample) then only 100 need to be staged and the rest apportioned across the stages found in that particular sample. If 100 eggs of the target species are **NOT** found in 1/4 or less of the sample then the whole sample will have to be sorted.

In some samples there might be large numbers of fish eggs present but relatively few eggs of the target species. In these cases the smaller eggs can be sub-sampled and the larger eggs sorted from the remainder of the sample. It is useful to make a glass pipette of a known aperture (e.g. 1.0mm diam.) and then any eggs that will not go into the pipette should be sorted from the sample for identification under a microscope. All larvae should be identified unless there are so much that sub-sampling is really necessary.

9.5 Storage of plankton samples (post analysis).

Sorted eggs and larvae should be placed in small glass vials containing preservative and stored in the larger jars used to hold the remainder of the sample to avoid wasting time re-sorting material if re-analysis is required.

Although the 'observation fluid' (see B2 above), as recommended by Steedman, 1976, does act as a preservative, it is recommended that a small amount of formaldehyde is added to this solution once each sample has been processed. To make up 10 litres of a stock solution for long term storage of the samples, the following quantities of chemicals are recommended.

50cm^3 Propylene phenoxetol

500cm^3 Propane-1, 2-diol

700cm^3 30% formaldehyde

This will give a final concentration of approximately 2% formaldehyde in the 'preservative' solution. This will prevent any deterioration of the plankton samples for a number of years.

Once the samples are in the 'preservative' solution it is recommended that the jars are dipped in molten wax to prevent evaporation from around the lid.

Responsibility for archiving samples will rest with the institutes that collected the samples.

9.6 Work up of additional measurements

PGEGGS agreed that the responsibility for work up of any additional measurements undertaken during PLACES would rest with the institute which had made the said measurements i.e. the PLACES consortium will only be directly responsible for work up of the core measurements within the program.

9.7 Work up of core data

Given the urgent policy and scientific drivers behind the call for the co-ordinated ichthyoplankton survey in the North Sea, PGEGGS agreed that it is very important that the results of PLACES be made available to ICES and end-users as rapidly as possible. Ideally the final data report would be completed by June 2005. This aim however has implications for additional resources required (see section 9.11)

The consortium agreed that plankton data from each institute would be compiled into a central ACCESS database. CEFAS offered to undertake this task on the condition that data are entered using CEFAS' standard software. CEFAS have an existing data handling system and its use will ensure efficient and rapid database construction. These procedures will be demonstrated at the autumn co-ordination meeting so that all participants can fully understand what they are agreeing to.

The database will be compiled as data are supplied to CEFAS and up-to-date version sent to participants on a three monthly basis. CEFAS will explore the possibility of making the database accessible via the internet to participants.

The CEFAS zooplankton database structure currently holds summary hydrographic information and is not linked to full individual station profiles. PGEGGS may require this facility so DIFRES will investigate how this can be achieved using software developed under the LIFECO program and report their findings to the autumn co-ordination meeting.

Individual institutes will be responsible for pre-processing hydrographic data via their standard protocols and for complying with national data handling requirements (for example CEFAS data require to be lodged with the British Oceanographic Data Centre). As well as supplying the summary statistics required for the zooplankton database (to CEFAS), individual institutes will pass hydrographic profiles from vertical CTD casts to the ICES hydrographic database.

Data will be analyzed using a variety of exploratory and spatial statistical methods which may include symbol based mapping, geostatistics, quantile regression and generalised additive modelling.

An agreement on data ownership will be made at the autumn 2003 co-ordination meeting.

9.8 Reporting of findings

The first reporting of findings will be through cruise reports, prepared within a month from the end of each cruise. The cruise reports will follow the format of the national institutes, but a common spreadsheet format will be used for the presentation of station information. If a web-page is established the cruise reports will be available there. When samples have been processed and biological information is available, a detailed report of results will be produced, focused on the observed distribution pattern of target species. This report will be presented to ICES medio 2005. While parts of the sampling are integrated in other projects, additional information will be available from the reporting of these projects (German Globec, IHLS). Further, the observed distribution patterns of eggs/larvae and the results of the genetic probe technique and the analysis of physical/biological linkages will be presented in a number of peer-reviewed articles during 2005.

9.9 Additional resources required

PGEGGS have produced a sampling program for PLACES which is partially viable with exisitng resource commitments e.g. sea time. However, other participants need to apply for seatime and staff time. The outcome of these applications is not yet known.

We anticipate that national funding bodies will have to provide the majority of the additional resources but PGEGGS will also explore the possibility of funding from other sources such as the EU:

Table 9.9.1 Additional resources required for PLACES

Program co-ordinator	T&S
Neth. Inst. Fish. Res.	Sea time Staff time Consumables T&S Sampling gear
IMR	Sampling gear Consumables T&S
DIFRES	Sampling gear Funding for plankton sample analysis T&S
IFM	Sampling gear Staff time Consumables T&S
BFA	Staff time Consumables T&S
CEFAS	T&S Staff time
Genetic analysis	Funds for sub-contracting genetic analyses

10 PROGRAM MANAGEMENT

The proposed survey program is ambitious in scope and will require strong project management.

10.1 Program co-ordination

PGEGGS recommend that a project co-ordinator be appointed and provided with sufficient resources to undertake this task (see section 9.9).

10.2 Autumn 2003 co-ordination meeting

In autumn (approximately November) PGEGGS will reconvene to work out the details of the egg and larval surveys in 2004 (PLACES 2004). At that time the exact amount of funding and resources available for PLACES must be clear. The group will set up a detailed working programme including a list of (scientific) goals and a task list for each of the participating institutes. Furthermore the group will discuss the responsibilities and aims for publishing in peer-reviewed journals.

10.3 Training for surveys, exchange of expertise

Sorting plankton samples and the identification and staging of eggs and larvae (both onboard of the research vessels and in the laboratory) requires expertise. Furthermore, in an international programme including various laboratories a calibration exercise is necessary for quality assurance.

If funding permits, CEFAS offers to train (new) staff at the CEFAS plankton laboratory. This training includes identification and staging of eggs and larvae in fixed samples. Training may also be required in recognising cod-like eggs in a fresh sample. An option is to have 1 or 2 expert plankton-analysts join every first cruise of an institute. However the feasibility of this option depends on funding. The cheaper option is developing a detailed key that is sufficient to use onboard when pre-sorting the samples.

After the training and before the onset of plankton sorting of the PLACES 2004 samples, all technicians /scientists carrying out plankton analyses should meet for an inter-calibration exercise in determinations and staging of eggs and larvae.

11 CONCLUSIONS

PGEGGS participants Norway, Denmark, Great Britain, The Netherlands and Germany are confident that an internationally co-ordinated plaice and cod egg survey in the North Sea is possible in 2004

Ship time offered/requested by consortium members would allow surveying of the entire North Sea south of 62° N.

The survey will be as synoptic as possible whilst taking account of the progressive delay of spawning from South to North. PGEGGS has set up a sampling scheme that meets these criteria.

The consortium agreed on sampling gear to be used, analyses that will provide comparable data between surveys by separate institutes and the way forward in terms of project management.

Dr Fox offered to act as program co-ordinator subject to sufficient additional resources being made available to undertake this task

The consortium agreed to meet in the autumn 2003 to undertake detailed planning for the surveys in 2004.

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13 REFERENCES

Armstrong, M.J. 1997. Estimation of fish biomass in the Irish Sea by means of the annual egg production method. The Queen's University of Belfast, European Community Specific Program for Research, Technological Development and Demonstration in the Field of Agriculture and Fisheries:- 1991-1994, AIR3-CT94-2263.

Armstrong, M.J. 2002. Development and validation of egg-production based biomass estimates, using cod and plaice stocks in the Irish Sea. Department of Agriculture and Environmental Science, Contract Final Report, Commission of the European Communities, Directorate general for Fisheries, Contract 98/090, 256 pp.

Bannister, R.C.A., D. Harding & S.J. Lockwood. 1973. Larval mortality and subsequent year-class strength in the plaice (*Pleuronectes platessa* L.). pp. 21-37. *In:* J.H.S. Blaxter (ed.) The Early Life History of Fish: Proceedings of an International Symposium, Springer-Verlag, Berlin, Dunstaffnage MArine Research Laboratory, Oban, Scotland.

Bergman, M.J.N., H.W. van der Veer & J.J. Zijlstra. 1988. Plaice nurseries: effects on recruitment. Journal of Fish Biology 33: 201-218.

Beverton, R.J.H. & T.C. Iles. 1992. Mortality rates of O-group plaice (*Pleuronectes platessa* L.) dab (*Limanda limanda* L.) and turbot (*Scophthalmus maximus* L.) in European waters. Netherlands Journal of Sea Research 29: 49-59.

Boehlert, G.W. & B.C. Mundy. 1988. Roles of behavioural and physical factors in larval and juvenile fish recruitment to estuarine nursery areas. American Fisheries Society Symposium 3: 51-67.

Brander, K. 1994a. Spawning and life history information for North Atlantic cod stocks. International Council for the Exploration of the Sea, Rapport des recherches Collectives, 205.

Brander, K. & P.C.F. Hurley. 1992. Distribution of early-stage Atlantic cod (Gadus morhua), Haddock (*Melanogrammus aeglefinus*), and Witch flounder (*Glyptocephalus cynoglossus*) eggs on the Scotian shelf: A reappraisal of evidence on the coupling of cod spawning and plankton production. Canadian Journal of Aquatic Science 49: 238-251.

Brander, K.M. 1994b. The location and timing of cod spawning around the British Isles. Journal of Marine Science 51: 71-89.

Bromley, P.J. 2000. Growth, sexual maturation and spawning in central North Sea plaice (*Pleuronectes paltessa* L.), and the generation of maturity ogives from commercial catch data. Journal of Sea Research 44: 27-43.

Bromley, P.J. & L.T. Kell. 1999. Vertical migration and spatial distribution of pelagic 0-group gadoids (cod, haddock, whiting and Norway pout) prior to and during settlement in the North Sea. Acta Adriatica 40: 7-17.

Buchanan-Wollaston, H.J. 1923. The spawning of plaice in the southern part of the North Sea in 1913-1914. Fishery Investigation Series II, 2, 36 pp.

Campos, W.L. 1996. A comparitive study of the distribution of dab (*Limanda limanda* L.) and flounder (*Platichthys flesus* L.) larvae in the southeastern North Sea with notes on their sensory development, University of Hamburg, Hamburg. 125 pp.

Coombs, S.H., J.H. Nichols & C.A. Fosh. 1990. Plaice eggs (*Pleuronectes platessa* L.) in the southern North Sea: abundance, spawning area, vertical distribution, and buoyancy. Journal de Conseil International pour l'Exploration de la Mer 47: 133-139.

Creutzberg, F. 1978. Transport of marine organisms by tidal currents. *In:* N. Dankers, W.J. Wolff & J.J. Zijlstra (ed.) Fish and fisheries in the Wadden Sea, Balkema, Rotterdam, Netherlands.

Cushing, D.H. 1969. The regularity of the spawning season in some fishes. Journal de Conseil International pour l'Exploration de la Mer 33: 81-97.

Cushing, D.H. 1975. Marine ecology and fisheries. Cambridge University Press, Cambridge, England.

Daan, N. 1978. Changes in cod stocks and cod fisheries in the North Sea. Rapports et Proces-Verbaux des Reunions, Conseil Rapports et Proces-Verabaux des Reunions, Conseil Permanent International pour l'Exploration de la Mer 172: 39-57.

Dickson, R.R. & K.M. Brander. 1993. Effects of a changing windfield on cod stocks of the North Atlantic. Fisheries Oceanography 2: 124-153.

Economou, A.N. 1991. Food and feeding ecology of five gadoid larvae in the northern North Sea. Journal de Conseil International pour l'Exploration de la Mer 47: 339-351.

Edwards, R. & J.H. Steele. 1968. The ecology of 0-group plaice and common dabs at Loch Ewe. Journal of Experimental Marine Biology and Ecology 2: 215-238.

Fossum, P. & B. Ellertsen. 1994. Gut content analysis of first-feeding cod larvae (*Gadus morhua* L.) off Lofoten, Norway, 1979-1986. ICES Marine Science Symposium 198: 430-437.

Fox, C.J., B. Planque & C.D. Darby. 2000. Synchrony in the recruitment time-series of plaice (*Pleuronectes platessa* L.) around the United Kingdom and the influence of sea temperature. Journal of Sea Research 44: 159-168.

Gregory, R.S. & J.T. Anderson. 1997. Substrate selection and use of protective cover by juvenile Atlantic cod *Gadus morhuai in inshore waters of Newfoundland*. Marine Ecology Progress Series 146: 9-20.

Harden Jones, F.R., G.P. Arnold, M. Greer Walker & P. Scholes. 1979. Selective tidal stream transport and the migration of plaice (*Pleuronectes platessa* L.) in the southern North Sea. Journal de Conseil International pour l'Exploration de la Mer 38: 331-337.

Harding, D. & J.H. Nichols. 1987. Plankton surveys off the north-east coast of England in 1976: an introductory report and summary of results. Directorate of Fisheries Research, Fisheries Research Technical REport, 86, 55 pp.

Harding, D., J.H. Nichols & D.S. Tungate. 1978. The spawning of plaice (*Pleuronectes platessa* L.) in the southern North Sea and English Channel. Rapports et Procès-verbaux des Réunions Conseils International pour l'Exploration de la Mer 172: 102-113.

Harding, D. & J.W. Talbot. 1973. Recent studies on the eggs and larvae of the plaice (*Pleuronectes platessa* L.) in the southern Bight. Rapports et Procès-verbaux des Réunions Conseils International pour l'Exploration de la Mer 164: 261-269.

Heath, M., P. Rankine & L.H. Cargill. 1994. Distribution of cod and haddock eggs in the North Sea in 1992 in relation to oceanographic features and compared with distributions in 1952-1957. ICES Marine Science Symposium 198: 438-439.

Heessen, H. & N. Daan. 1994. Cod distribution and temperature in the North Sea. ICES Marine Science Symposium 198: 244-253.

Heessen, H.J.L. 1993. The distribution of cod (*Gadus morhua*) in the North Sea. NAFO Sci. Coun. Studies 18: 59-65.

Heessen, H.J.L. & A.D. Rijnsdorp. 1989. Investigations on egg production and mortality of cod (*Gadus morhua* L.) and plaice (*Pleuronectes platessa* L.) in the southern and eastern North Sea in 1987 and 1988. Conseil Rapports et Proces-Verabaux des Reunions, Conseil Permanent International pour l'Exploration de la Mer 191: 15-20.

Hill, A.E. 1991. Vertical migration in tidal currents. Marine Ecology Progress Series 75: 39-54.

Horwood, J.W., R.C.A. Bannister & G.J. Howlett. 1986. Comparitive fecundity of North Sea plaice (*Pleuronectes platessa* L.). Proceedings of the Royal Society London 228 B: 401-431.

Houghton, R.G. & D. Harding. 1976. The plaice of the English Channel: spawning and migration. Journal de Conseil International pour l'Exploration de la Mer 36: 229-239.

ICES. 2003. Report of the Regional Ecosystem study Group for the North Sea. International Council for the exploration of the Seas, CM, CM 2003/ACE:04, 34 pp.

Koutsikopoulos, C., L. Fortier & J.A. Gagne. 1991. Cross-shelf dispersion of Dover sole (Solea solea) eggs and larvae in Biscay Bay and recruitment to inshore nurseries. Journal of Plankton Research 13: 923-945.

Last, J.M. 1980. The food of twenty species of fish larvae in the west-central North Sea. Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, 44 pp.

McLaren, I.A. & P. Avendano. 1995. Prey field and diet of larval cod on Western Bank, Scotian Shelf. Canadian Journal of Fisheries and Aquatic Sciences 52: 448-463.

McLaren, I.A., P. Avendaño, C.T. Taggart & S.E. Lochmann. 1997. Feeding by larval cod in different water-masses on western Bank, Scotian Shelf. Fisheries Oceanography 6: 250-265.

Meeren, T.V.d. 1991. Algae as first food for cod larvae, *Gadus morhua* L.: filter feeding or ingestion by accident? Journal of Fish Biology 39: 225-237.

Metcalfe, J.D. & G.P. Arnold. 1997. Tracking fish with electronic tags. Nature 387: 665-666.

Mork, J., P. Solemdal & G. Sundes. 1983. Identification of marine fish eggs: A biochemical genetics approach. Canadian Journal of Fisheries and Aquatic Sciences 40: 361-369.

Munk, P. 1997. Prey size spectra and prey availability of larval and small juvenile cod. Journal of Fish Biology 51: 340-351.

Munk, P., P.O. Larsson, D. Danielsen & E. Moksness. 1995. Larval and small juvenile cod *Gadus morhua* concentrated in the highly productive areas of a shelf break front. Marine Ecology Progress Series 125: 21-30.

Munk, P., P.O. Larsson, D. Danielsen & E. Moksness. 1999. Variability of frontal zone formation and distribution of gadoid fish larvae at the shelf break in the northeastern North Sea. Marine Ecology Progress Series 177: 221-233.

Munk, P., P.J. Wright & N.J. Pihl. 2002. Distribution of the early larval stages of cod, plaice and lesser sandeel across haline fronts in the North Sea. Estuarine and Coastal Marine Science 55: 139-149.

Nash, R.D.M., M. Dickey-Collas & S.P. Milligan. 1998. Descriptions of the Gulf VII/PRO-NET and MAFF/Guildline unencased high-speed plankton samplers. Journal of Plankton Research 20: 1915-1926.

Nichols, J.H. 1989. The diurnal rhythm in spawning of plaice (*Pleuronectes platessa L.*) in the southern North Sea. Journal de Conseil International pour l'Exploration de la Mer 45: 277-283.

O'Brien, C.M. & C.J. Fox. 2000. Accounting for spatial-scale and temporal information in ichthyoplankton surveys using a model-based approach: cod (*Gadus morhua* L.) in the Irish Sea. ICES, CM 2000, K:18, 11 pp.

Planque, B. & T. Frédou. 1999. Temperature and the recruitment of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries and Aquatic Sciences 56: 2069-2077.

Raitt, D.F.S. 1967. Cod spawning in Scottish waters. Preliminary investigations. Internation Council for the Exploration of the Sea, C.M., 1967/F:29, 3 + 13 Figures pp.

Righton, D., J. Metcalfe & P. Connolly. 2001. Different behaviour of North and Irish Sea cod. Nature 411: 156.

Rijnsdorp, A.D. 1989. Maturation of male and female North Sea plaice. Journal de Conseil International pour l'Exploration de la Mer 46: 35-51.

Rijnsdorp, A.D. 1991. Changes in fecundity of female North Sea plaice (*Pleuronectes platessa* L.) between three periods since 1980. ICES Journal of Marine Science 48: 253-280.

Rijnsdorp, A.D., M.v. Stralen & H.W. van der Veer. 1985. Selective tidal transport of North Sea plaice larvae *Pleuronectes platessa* in coastal nursery areas. Transactions of the American Fisheries Society 114: 461-470.

Rijnsdorp, A.D. & D. Vethaak. 1997. Changes of reproductive parameters of North Sea plaice and sole between 1960 and 1995. International Council for the Exploration of the Seas, CM, CM 1997/U:14.

Riley, J.D. & W.G. Parnell. 1984. The distribution of young cod. pp. 563-580. *In:* E. Dahl, D.S. Danielssen, E. Moksness & P. Solemdal (ed.) The Propagation of Cod *Gadus morhua* L. Flodevigen rapportster, 1, Institute of Marine Research, Arendal.

Riley, J.D., D.J. Symonds & L. Woolner. 1986. Determination of the distribution of the planktonic and small demersal stages of fish in the coastal waters of England, Wales and adjacent areas between 1970 and 1984. MAFF Directorate of Fisheries Research, Fisheries Research Technical Report, 108, 23 pp.

Riley, J.D., D.J. Symonds & L.E. Woolner. 1981. On the factors influencing the distribution of 0-group demersal fish in coastal waters. Rapports et Procès-verbaux des Réunions Conseils International pour l'Exploration de la Mer 178: 223-228.

Rogers, S.I., R.S. Millner & T.A. Mead. 1998. The distribution and abundance of young fish on the east and south coast of England(1981 to 1997). CEFAS, Science Series Technical Report, 108, 129 pp.

Russell, F.S. 1976. The Eggs and Planktonic Stages of British Marine Fishes. Academic Press, London. 524 pp.

Ryland, J.S. & J.H. Nichols. 1975. Effect of temperature on the embryonic development of the plaice, *Pleuronectes platessa* L.(Teleostei). Journal of Experimental Marine Biology and Ecology 18: 121-137.

Saville, A. 1959. The planktonic stages of the haddock in Scottish waters. Marine Research 3: 23.

Simpson, A.C. 1959. The spawning of the plaice (*Pleuronectes platessa*) in the North Sea. Ministry of Agriculture, Fisheries and Food, Fisheries Investigations, Series II, Vol. XXII, No. 7, 111 pp.

Simpson, A.C. 1971. Diel spawning behaviour in populations of plaice, dab, sprat and pilchard. Journal de Conseil International pour l'Exploration de la Mer 34: 58-64.

Sundby, S. 1991. Factors affecting the vertical distribution of eggs. ICES Marine Science Symposium 192: 33-38.

Sundby, S. 2000. Recruitment of Atlantic cod stocks in relation to temperature and advection of copepod populations. Sarsia 85: 277-298.

Talbot, J.W. 1977. The dispersal of plaice eggs and larvae in the Southern Bight of the North Sea. Journal de Conseil International pour l'Exploration de la Mer 37: 221-248.

Taylor, M.I., C.J. Fox, I. Rico & C. Rico. 2001. Species-specific TaqMan probes for simultaneous identification of cod (*gadus morhua* L.), haddock (*Melanogrammus aeglefinus* L.) and whiting (*Merlangius merlangus* L.). Molecular Ecology Notes 2: 599-601.

Thompson, B.M. & J.D. Riley. 1981. Egg and larval development studies in the North Sea cod (*Gadus morhua* L.). Rapports et Procès-verbaux des Réunions Conseils International pour l'Exploration de la Mer 178: 553-559.

Van Beek, F.A., A.D. Rijnsdorp & R. De Clerk. 1989. Monitoring juvenile stocks of flatfish in the Wadden Sea and coastal areas of the southeastern North Sea. Helgolander Meeresuntersuchungen 43: 461-477.

van der Land, M.A., H.J.L. Heessen & A.D. Rijnsdorp. 1990. The results of the 1989 egg surveys for cod and plaice. International Council for the Exploration of the Seas, C.M., C.M. 1990/G:27, 14 pp.

van der Veer, H. & J.I.J.m. Witte. 1999. Year-class strength of plaice *Pleuronectes platessa* in the Southern bight of the North Sea: a validation and analysis of the inverse relationship with winter seawater temperature. Marine Ecology Progress Series 184: 245-257.

van der Veer, H.W. 1986. Immigration, settlement, and density-dependent mortality of a larval and early postlarval O-group plaice (*Pleuronectes platessa*) population in the western Wadden Sea. Marine Ecology Progress Series 29: 223-236.

van der Veer, H.W., I. Pihl & M.J.N. Bergman. 1990. Recruitment mechanisms in North Sea plaice *Pleuronectes platessa*. Marine Ecology Progress Series 64: 1-12.

van der Veer, H.W., P. Ruardy, A.J. van der Berg & H. Ridderinkhof. 1998. Impact of interannual variability in hydrodynamics circulation on egg and larval transport of plaice *Pleuronectes platessa* L. in the southern North Sea. Journal of Sea Research 39: 29-40.

van der Veer, H.W. & J.I.J. Witte. 1993. The 'maximum growth/optimal food condition' hypothesis: a test for O-group plaice *Pleuronectes platessa* in the Dutch Wadden Sea. Marine Ecology Progress Series 101: 81-90.

Weidman, C.R. & R. Millner. 2000. High-resolution stable isotope records from North Atlantic cod. Fisheries Research 46: 327-342.

Zijlstra, J.J. 1972. On the importanfce of the Wadden Sea as a nursery area in relation to the conservation of the southern North Sea fishery resources. Symposium of the Zoological Society of London 29: 233-258.

Zijlstra, J.J., R. Dapper & J.I. Witte. 1982. Settlement, growth and mortality of post-larval plaice (*Pleuronectesplatessa*) in the Western Wadden Sea. Netherlands Journal of Sea Research 15: 250-272.